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DAMPING-OFF IN FOREST NURSERIES

A THESIS ACCEPTED IN PARTIAL SATISFACTION OF
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CARL HARTLEY

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Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER June 16, 1921

DAMPING-OFF IN FOREST NURSERIES

By

CARL HARTLEY, formerly Pathologist, Office of Investigations in Forest Pathology

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DAMPING-OFF IN GENERAL.

Damping-off is the commonest English name for a symptomatic group of diseases affecting great numbers of plant species of widely separated phylogenetic groups. It is commonly used for any disease which results in the rapid decay of young succulent seedlings or soft cuttings. Young shoots from underground rootstocks may also be damped-off before they break through the soil (66).[1] The same term is even used for diseases affecting the prothallia of vascular cryptogams (2). The name apparently originated in the fact that the disease is usually most prevalent under excessively moist conditions. In those cases in which the disease becomes serious without the presence of unusual amounts of moisture the term is a misnomer. It is, however, so thoroughly established in practical use that it would be impossible, even if desirable, to establish any other name.

[1] The serial numbers in parentheses refer to "Literature cited," at the end of this bulletin.

While the parasites reported as causing damping-off are probably not as numerous as the host species which are subject to it, a considerable number are known. Two quite different types of damping-off parasites may be recognized. In the first type we have fungi, such as *Pythium debaryanum* Hesse and *Corticium vagum* B. and C., soil inhabiting and primarily saprophytic, which attack a great variety of hosts, and are at least better known, if not more destructive, as damping-off organisms than as parasites on older plants. They are specialized as to the type and age of tissues which they attack rather than as to host. The second type includes fungi less common as saprophytes and with a relatively limited, sometimes very closely limited, host range. *Phoma betae*, the systemic parasite of sugar beet (37), is an excellent example of the host-specialized parasite, transmitted in the seed and capable of seriously injuring various parts of the older plant at different stages of growth as well as attacking seedlings.

Most damping-off parasites are intermediate in habit between the extremes of these two types. Of those which are somewhat host specialized, the following may be mentioned:

Phomopsis vexans, the cause of foot-rot of eggplant, reported by Sherbakoff (128) as a frequent cause of damping-off of this host and believed to be carried on seed.

Gibberella saubinetii (Mont.) Sacc. (29) and the imperfect fungi which kill grain seedlings as well as cause diseases of the older plants (80; 126, p. 218). Species of Gloeosporium and Volutella named by Atkinson (2, p. 269; 52) as able to kill seedlings or cuttings of particular host plants.

Glomerella (*Colletotrichum*) *gossypii*, described by Atkinson (1) and Barre (4) as likely to cause damping-off of cotton (112).

Fusarium lini, the flax parasite, reported by Bolley (14) as destructive to young seedlings.

Phoma lingam, the cause of black-leg of cabbage, at least under inoculation conditions able to kill quickly seedlings of cabbage and other crucifers (72).

Peronospora parasitica (Pers.) De Bary, a downy mildew attacking cabbage and various other crucifers, reported as killing thousands of very young cabbage plants in Florida seed beds (41).

The entomophthoraceous *Completoria complens*, on fern prothallia (1; 87, p. 203).

Bacillus malvacearum, a parasite of the leaves of cotton plants, which can also cause damping-off of its favorite host (113) and the bacteria from diseased cucumber plants with which Halsted (53) caused typical damping-off of cucumbers.

Damping-off fungi with wider host ranges include *Phytophthora fagi*, *Aphanomyces levis* (100), *Rheosporangium aphanidermatus* (38, 39), *Botrytis cinerea*, and certain Fusaria. The so-called propagation fungus, "vermehrungspilz," a sterile damping-off mycelium which Sorauer (133, p. 321) believed related to Sclerotinia and for which Ruhland (115) has erected a new genus, considered by both

authors the most serious enemy encountered in growing softwood cuttings in Germany, if distinct would be a further addition to these generalized parasites. However, it is now believed (34) to be identical with *Corticium vagum*. Common generalized parasites of older plants, such as *Sclerotinia libertiana*, *Sclerotium rolfsii* (129), and *Thielavia basicola* (47), capable of attacking roots or other parts of older plants of numerous species, may also be considered among the damping-off fungi when they cause the death of small seedlings, as occurs, for example, in attacks by *Sclerotinia libertiana* on lettuce (20, p. 28) and celery (103, p. 536) in seed beds. Further study will probably result in multiplying almost indefinitely the number of more or less important damping-off parasites, both of the specialized and unspecialized groups, although the most important of the latter type are probably already known.

Most of the references in literature to damping-off describe its occurrence in truck crops and the losses caused in these crops. According to Halsted (53, p. 342), weed seedlings are also very commonly attacked. Duggar (33) names lettuce, celery, cotton, sugar beet, cress, cucumber, and sunflower as especially susceptible to injury by the two most important damping-off organisms. Except for the plant species in which damping-off by seed-carried parasites is common, it appears that the economic damage from damping-off is serious only with plants whose culture involves the raising of the seedlings in crowded seed beds for subsequent transplanting. For example, tomatoes do not ordinarily suffer from damping-off in the field (70), but the growing of seedlings in flats for subsequent transplanting is sometimes seriously hampered as a result of the prevalence of damping-off. This same principle holds in general for trees. Broad-leaved trees, which are usually not as crowded in the seedling stage as are the conifers, seldom give rise to complaint on the score of damping-off. The conifers, subject to serious losses in nursery beds, are not believed to be greatly affected in this country by the better known types of damping-off under forest conditions (68) except in the less common cases in which seedlings come up in close groups from squirrel hoards, artificial seed spots, or similar sources.

A considerable number of broad-leaved trees have been reported at one time or another as injured by damping-off, though complaints of commercially serious losses are not common. The cases which have come to the writer's attention are listed below:

Cause not determined:

Orange (43, 108).

Olive, in greenhouse at the University of California.

Russian wild olive (*Elaeagnus* sp.), serious at an Iowa nursery; oral report by Mr. C. R. Bechtle, formerly of the United States Forest Service; at another nursery in the same region this plant was reported as very little subject to injury.

Cause not determined—Continued.

Magnolia (31), troublesome if the pulp is not washed off the seed before planting.

Eucalyptus spp. (88, p. 45; 131), serious under moist conditions.

Betula spp. Communication by Dr. Perley Spaulding, of the Bureau of Plant Industry; found especially susceptible in a Pennsylvania nursery.

Carob, at United States Plant Introduction Garden, Chico, Calif. Dr. Mel T. Cook states that damping-off is more serious in carob seedlings if the seed is removed from the pod than if pods and seeds are sown together.

Robinia pseudacacia (13).

Apple, in greenhouse at the Michigan Agricultural College.

Sclerotinia sp. (Europe):

Betula (79), a disease of seed and germinating seedlings.

Phytophthora fagi (Europe):

Fagus. Hartig (59) and many other writers; seriously affected, even in forest.

Platanus (15).

Acer (15), *A. platanoides* and *A. pseudoplatanus* (86, 104).

Robinia (59, 73).

Fraxinus (73).

Acacia (59).

Cercospora acerina (Europe):

Acer platanoides and *A. pseudoplatanus* (58).

Pythium debaryanum:

Tilia europea and *T. ulmifolia* (137), serious.

Robinia (75, p. 13–14), killing germinating seed.

Catalpa (126).

Rhizoctonia:

Citrus seed beds (130); much loss.

Catalpa (126).

Botrytis cinerea:

Catalpa (126).

Fusarium sp.:

Citrus seed beds (130); much loss.

The sugar beet is apparently the only plant whose damping-off diseases have been investigated with any degree of completeness by modern methods. While there is a great mass of literature on damping-off, it is mainly descriptive and on control measures. Most of the reports of the causal relation between the different fungi and the disease in the various host plants have been based on demonstrations of the presence of the fungus in diseased seedlings. In a great number of these cases identification has been doubtful. Even when a fungus is known to belong to a parasitic species, it is by no means certain that the mycelium found belongs to a parasitic strain. It has been found, for example, that only part of the strains of *Corticium vagum* occurring in sugar beets are able to attack that host vigorously (38, p. 154). Similar data for pine appear in figures 1 and 2. Furthermore, even parasitic strains of several of the damping-off organisms are so widely distributed as

saprophytes that one of them might easily get into a killed seedling after some other parasite had caused its death. Not only in the case of seedlings killed by fungi like *Peronospora parasitica*, but in

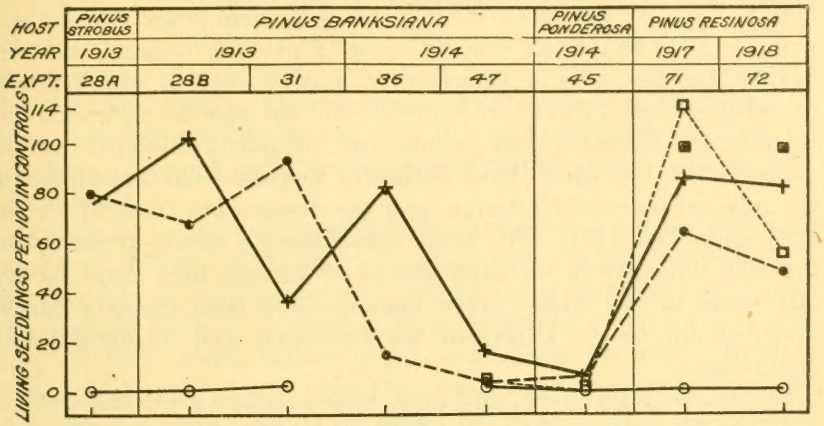

FIG. 1.—Diagram showing the relative activity of different strains of *Corticium vagum* in inoculations made at the time of sowing the seed. In experiments Nos. 36, 45, and 47 the values are plotted for the number of seedlings appearing above the soil. For the other experiments the number of seedlings surviving at the close of the experiment have been taken. Explanation of symbols: ○=Strain 147, from spruce seedlings, Washington, D. C., 1910; ✚=strain 50, from pine seedlings, Nebraska, 1909; □=strain 233, from *Elaeagnus* sp., Kansas, 1913; ■=strain 230, from the same lesion as strain 233; ●=strain 183, from bean, New York, 1910.

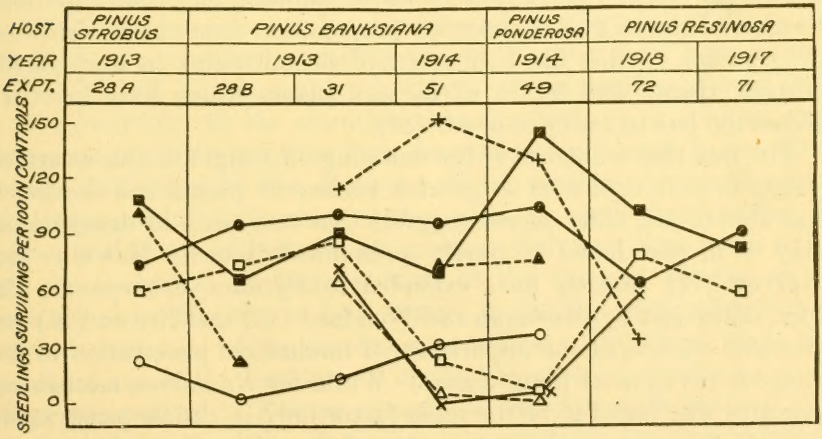

FIG. 2.—Diagram showing the relative activity of different strains of *Corticium vagum*, as indicated by the number of seedlings surviving in inoculated soil. Explanation of symbols: ●=Strain 189, from sugar beet, Michigan, 1910 (light-brown mycelium with few sclerotia); ▲=strain 211 and △=strain 212, from sugar beet, Colorado, 1910; ■=strain 186, from potato, Ohio, 1910; □=strain 187, from potato, New York, 1910; ✚=strain 205, from Douglas fir, Colorado, 1911; ×=strain 192 and ○=strain 206, from pine, Nebraska, 1911.

cases of true damping-off produced by the rotting type of parasite, much of the rapid decay of the seedling after death is brought about by bacteria and fungi other than the one causing death.

Inoculation experiments are therefore probably even more necessary in damping-off investigations than in studies of most other diseases in order to demonstrate etiological relationships. Unfortunately, most of the inoculation work with damping-off organisms prior to 1900 was either crudely done by placing diseased seedlings against healthy ones or consisted of experiments in which purity of cultures and validity of controls did not receive sufficient consideration. Recent investigations not primarily directed toward damping-off, but which have decidedly increased our knowledge of the relation between Corticium and the disease, are those of Peltier (98) and Fred (43). The latter established a strong presumption that the difficulty in securing stands of various field crops having oily seeds in soil where green manures had been recently turned under is due to the killing of the sprouting seed by damping-off organisms.

In tobacco, sugar beet, and pine, whose damping-off has received considerable attention, it has been found that the damping-off proper is commonly preceded by the killing of many of the sprouting seeds in the soil (38; 68, p. 522; 81, p. 5) and followed, after the plants become too large to be killed by the damping-off organisms, by root sickness and the death of small roots (38, p. 161; 64; 100). This latter has been reported also as a serious matter in the case of *Corticium vagum* for potato (51), a host on which damping-off is not important because of the lack of commercial propagation from seed. *Pythium debaryanum* further has been reported as continuing to work in the cortical tissues and leaves of tobacco plants which have been infected too late to result in death (81).

The fact that a number of the damping-off fungi are able to attack young or soft tissues of so great a variety of plants and are much less able to kill older plants suggests that resistance to damping-off may be in part based on purely mechanical factors. Hawkins and Harvey (71) recently have extended to *Pythium debaryanum* the idea, developed by Blackman and Welsford (12) and Brown (16) for *Botrytis cinerea*, of the importance of mechanical penetration in the fungous invasion of plant tissues. While for *B. cinerea* mechanical pressure was found to be the main factor only in cuticle penetration, with *P. debaryanum* the penetration of the cell walls of all parts of the potato tuber was apparently largely dependent on mechanical puncturing by the hyphæ, only tubers with mechanically weak cell walls being susceptible to decay by the fungus. The extreme susceptibility to *P. debaryanum* and *Corticium vagum* of soft, thin-walled tissues and the resistance of older stems and root parts would fit in well with such a theory as to the method of wall penetration, as in the older tissues the thicker cell walls would obviously be a serious bar to the extension of a fungus dependent partly or en-

tirely on mechanical puncturing for its progress from cell to cell. Hartig (61, p. 147–150) shows a fungus which he does not name, but which is evidently a species of Fusarium, dissolving the young uncuticularized epidermis of pine seedlings; but he states that it can not so dissolve older epidermis. The increased protective value of the epidermis of older plants can only in part explain the immunity most of them develop against serious attack by damping-off organisms, as lesions already started or which may later develop from the infection of young roots are unable to extend into the older parts of the plants.

It may be mentioned here that the writer in a very preliminary test found strains of *Corticium vagum* and *Fusarium moniliforme* Sheldon which had been proved able to cause damping-off of pines also apparently able to destroy filter paper in inorganic salt solution, while *Pythium debaryanum* seemed not so able. Ruhland (116), on the other hand, found the strain of the "vermehrungspilz" (*Corticium vagum*) which he tested to be very weak in cellulose-destroying ability as compared with *Botrytis cinerea*.

DAMPING-OFF OF CONIFERS.

HISTORICAL.

While the losses from damping-off in seed beds of dicotyledonous tree species are occasionally serious and in the case of beech in Europe have required considerable study, they have been so far overshadowed in this country by the losses in coniferous seed beds that practically all the attention thus far, both as to etiology and measures of prevention, has been devoted to the disease in conifers.

The literature on the damping-off of conifers is considerable. A large part of it, because of the extensive early development of plant pathology and forest planting in Germany, has been written by Germans. A large portion of the German articles on it was either by foresters or by botanists in the day when most pathological work was of the reconnaissance type. Therefore, while the work of one of the best known of the parasites on coniferous seedlings was noticed in Europe as early as the eighteenth century (21, p. 252–253) most of the European data available are observational. The only fungi which were at all definitely connected with the disease on conifers seem to have been Fusarium (*Fusoma* spp.) and *Phytophthora fagi* (*P. omnivora* De Bary in part). The damping-off Rhizoctonia was described in Germany in 1858 and *Pythium debaryanum* in 1874; the fact that neither of these, important in coniferous seed beds in both the eastern and western United States, has ever been reported from conifers in Europe is perhaps the best evi-

dence of the relatively small amount of actual investigation carried on there on this disease in the nurseries. A number of references to the damping-off of conifers in the English horticultural and botanical literature yield even less definite information as to the causal fungi than do the German articles.

With the awakening of interest in reforestation in the United States between 15 and 20 years ago and the first efforts to grow pines in quantity for forestry purposes, attempts were made to determine the cause of the disease in this country and to develop direct-control methods. Duggar and Stewart (32) made what appears to be the first report of Rhizoctonia in connection with the damping-off of conifers. Spaulding (136, 137), in work begun in 1905, contributed much to our knowledge of the etiology of the damping-off of pine in this country, especially in relation to Fusarium, and originated the sulphuric-acid method of control. The writer in 1910 reported preliminary inoculations on conifers with both Rhizoctonia and *Pythium debaryanum* (62). The work of Gifford (46) and Hofmann (77) added to the information on the causal relation of *Fusarium* spp. and *P. debaryanum*, respectively. Hartley, Merrill, and Rhoads (68) have recently established the parasitism of a number of strains of the *Corticium vagum* type of Rhizoctonia on pine seedlings under inoculation conditions, have confirmed Spaulding's conclusions as to the parasitism of *Fusarium moniliforme* Sheldon, and have given preliminary data on other fungi. They consider *P. debaryanum* and *C. vagum* more important in pine seed beds than any single Fusarium species. Hartley and Hahn (69) have announced successful inoculations on pines with *P. debaryanum* and *Rhcosporangium aphanidermatus* Edson, with less satisfactory evidence of the parasitism of *Phytophthora* sp. and a fungus tentatively referred to *Pythium artotrogus*. Hartley and Pierce (67) report the finding of *P. debaryanum* in *Tsuga mertensiana* and *Pseudotsuga taxifolia* as well as in the pines. In damped-off pine seedlings they find *P. debaryanum* more commonly than *C. vagum*, especially in beds which have received disinfectant treatments. Other considerations, however, keep them from concluding that the former is necessarily the more important of the two. Both of these latter papers and all of the reports of Pythium with the exception of Hofmann's are brief notes, presenting no evidence in support of the statements made.

DESCRIPTION.

The symptoms of damping-off in conifers have already been described in some detail (68). In the paper cited, injury due to excessive heat of the surface soil and injury caused by high wind, both of which may easily be confused with damping-off, are described and accompanied by colored illustrations both of different types of damp

ing-off and of these nonparasitic troubles. The detailed descriptions will not be repeated here. A brief summary of the different types of disease recognized as included in damping-off follows:

(1) Germination loss: The radicles are killed very soon after the seeds sprout and before the seedlings can appear above ground. This is an important type, which can be caused probably by any of the organisms commonly capable of causing the better known types of trouble (61, 63, 68, 137).

(2) Normal damping-off (figs. 3, 4, and 5): The seedlings are killed by fungi invading either the root or hypocotyl after the seedling has appeared above the soil and while the stem is still dependent largely on the turgor of its cortical tissues for support. In sandy soils root infection is more common than hypocotyl infection, though the latter is the type most emphasized in the early horticultural descriptions. Büttner (26) some time ago recognized the frequence of

FIG. 3.—Normal type of damping-off of *Pinus ponderosa*. At the left is a damped-off seedling or root sprout of the southwestern ragweed (*Ambrosia psilostachya*). (Photographed by S. C. Bruner.)

root infections. Damping-off in beds out of doors is primarily in most cases a root rot, either of this type or of the types preceding and following.

(3) Late damping-off includes cases of the root-rot type occurring only after the seedling stems have started to become woody and the cortex has begun to shrivel. The damping-off parasites, or at least part of them, continue to kill seedlings by rotting their roots for some time after the stems become too woody to be decayed. The seedlings affected do not fall over till a considerable time after death. For convenience, all cases of this sort up to the purely arbitrary age of two months are classed as damping-off. However, in weather permitting of average speed of development the seedlings are usually able to resist attack before they reach this age. Seedlings at the marginal age between susceptibility and nonsusceptibility to killing infections are found often with the younger parts of their roots killed, but with the older portions apparently able to resist invasion by the fungus, recovery taking place by laterals. Dr. R. D. Rands and the writer in 1911 established the ability of seedlings from 43-day-old beds of *Pinus sylvestris*, *P. banksiana*, *P. nigra austriaca*, and *P. nigra poiretiana* to survive such infections, even when more than half of the root system has been destroyed, by transplanting such root-sick seedlings and

observing their continued growth (fig. 6). An article recently found (25) shows that Büttner had earlier made the same sort of demonstration of recovery of root-sick conifers. Observations on olive seedlings in 1916 showed cases of partially rotted roots which were recovering by sending out lateral root branches.

(4) Top damping: The cotyledons or upper part of the stem are invaded by the parasite, sometimes before the seedling breaks through the soil. The infection may or may not be fatal. A special case of this type, probably caused by a different parasite from those most commonly active, is that which in a publication above referred to was described and figured as "black-top" (68). It is

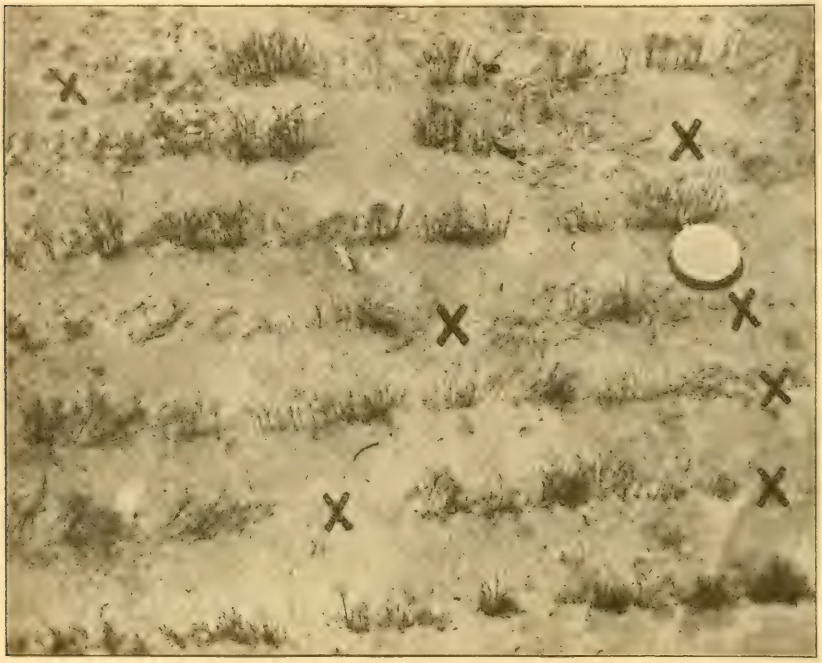

FIG. 4.—The beginning of an epidemic in drill-sown *Pinus banksiana*. Black crosses (X) indicate disease foci where the germinating seed were apparently killed and from which the disease is now spreading to adjacent seedlings. (Photographed by Dr. J. V. Hofmann.)

distinguished from ordinary top damping by the very dark color of the invaded tissues and its apparent dependence on some unusual set of climatic factors for its progress in the seedling after infection.

The killing of dormant seed by fungi is a matter of some practical interest in seed beds, and possibly still more so in forests, as it may help to explain the failure of certain conifers to reproduce except on mineral or certain other special soil types (68). With sugar beets *Pythium debaryanum* (100) is said to attack dormant seed as well as seeds which have sprouted. It is to be presumed that with conifers some of the damping-off fungi will be found to attack dormant as well as sprouting seed. This matter is now under investigation.

Something is already known about the seed fungi of herbaceous plants (76, 91), broad-leaved trees (79, 92), and juniper (95).

RELATIVE IMPORTANCE OF THE DIFFERENT TYPES.

Of the types of damping-off described in the foregoing pages the first two are ordinarily the most important. Late damping-off is rarely as serious as the normal type of damping-off. Top damping is only of importance in cases of excessive and unusual atmospheric moisture, so far as the writer's experience indicates. In the Middle West it has proved relatively insignificant. The three types which

FIG. 5.—Nearly complete destruction of the seedlings of *Pinus banksiana* at an unusually early age, at Garden City, Kans. (Photographed by Dr. J. V. Hofmann.)

occur after the seedlings appear above the soil surface can, of course, be evaluated by frequent counts during the damping-off season. This has apparently not yet been done by anyone. However, in experiments on damping-off control by soil disinfection, data have been obtained on comparative emergence (number of seedlings appearing above the soil surface) in treated and untreated plats and on the total parasitic losses after the seedlings appear which permit a certain amount of analysis of the losses due to damping-off parasites. The data from five nurseries bearing on this point are presented in Table I.

TABLE I.—*Relative importance of losses by damping-off before and after conifer seedlings emerge from the soil.*

Nursery and species.	Series.	Basis.			Loss in control plats.			
		Disinfectant.	Number of plats.		Emerged (viable seeds).			Ratio of col. 6 to col. 7.
			Treated.	Controls.	Before.	After.	Total.	
1	2	3	4	5	6	7	8	9
Bessey (Nebraska sand hills):					P. ct.	P. ct.	P. ct.	
Pinus banksiana	Average of 9	Sulphuric acid	(a)	(b)	37.8	c27.2	65.0	c1.39
Pinus ponderosa	Average of 2	do	6	8	28.1	27.3	55.4	1.03
Pinus resinosa	Average of 3	do	4	7	29.4	54.1	83.5	.54
Garden City (southwestern Kansas):								
Pinus austriaca	Average of 2	Copper sulphate.	4	3	69.0	27.5	96.5	2.52
Pinus banksiana	do	Zinc chlorid	3	2	80.7	12.0	92.7	6.72
Pinus ponderosa	Average of 7	Copper sulphate.	17	29	15.3	30.9	46.2	.49
Cass Lake (northern Minnesota):								
Pinus resinosa	No. 1051	Formaldehyde	6	3	3.8	37.2	41.0	.10
	No. 1052	do	6	3	25.7	36.2	61.9	.71
	No. 1053	Zinc chlorid	4	4	5.7	26.2	31.9	.22
	No. 1054	do	4	3	7.4	43.6	5.1	.18
	Nos. 1057 and 1061.	Heat	4	2	4.9	16.9	21.8	.29
East Tawas (Michigan):								
Pinus resinosa	1073	Formaldehyde	6	3	5.9	45.3	51.2	.13
	1074	Sulphuric acid	2	7	58.2	18.0	76.2	3.25
Fort Bayard (New Mexico):								
Pinus ponderosa	Nos. 791 and 792.	Formaldehyde	7	8	12.6	36.1	48.7	.35
	Nos. 891 and 892.	Sulphuric acid	8	6	14.5	18.6	33.1	.78

[a] Area counted, 122 square feet. [b] Area counted, 78 square feet.
[c] In *Pinus banksiana* at the Bessey Nursery, the loss after emergence is slightly low and the ratio slightly high, because of the closing of counts on a few of the series before damping-off was entirely over.

The procedure was to average the number of seedlings which emerged in the control plats in each series and subtract this number from the average number emerging (that is, appearing above the soil surface) in the treated plats in the same series. The treated plats chosen were the ones which allowed the averaging of the greatest number of plats treated with the same disinfectant. Only those plats were taken in which there was no evidence of injury to the seed or seedlings by the disinfectant and in which the amount of normal damping-off during the first few days after emergence was so slight as to indicate satisfactory initial control of the parasites by the treatment. In such plats it was assumed that the germination loss was unimportant, and the average number of seedlings appearing on them was taken as representing the number of viable seeds per plat. The difference between this emergence figure and the average emergence in the controls was taken as indicating the extent of parasitic loss before the seedlings appeared, including any destruction of dormant seed by parasites which may have occurred as well as the killing of germinating seed. Both this figure and the number

of seedlings which succumbed to damping-off after emergence were reduced to a percentage based on the indicated number of viable seeds, and they are directly compared in columns 6 and 7 of Table I. At three of the nurseries the data of the same species of pine and with the same treatment were averaged.

The data in Table I do not indicate any regularity either in the extent of loss before emergence, the loss after emergence, or in the ratio between these two values. For obvious reasons, no regularity is to be expected in any of these items. The table is of some interest, however, in confirming the evidence of the inoculation experiments, of observation of sprouting seed dug up in the beds, and of the partial or complete failure of emergence at the centers of large damping-off foci (figs. 4, 7, and 8) that the work of parasites before the seedlings appear may in some cases be of considerable importance. It is obviously impossible to make any general quantitative statement of the seriousness of such loss, in view of the variation in its extent at different times and places and of the inaccuracy of any computations based on the relative emergence of hosts as irregular in their germination as the conifers are known to be. The case is complicated in addition by the fact that, despite careful avoidance of treated plats known to have suffered chemical injury, it is probable that a few seedlings were killed before emergence by the disinfectants used in some of the

FIG. 6.—Root sickness in *Pinus nigra poiretiana*. The two seedlings at the right are healthy. The three at the left have had their taproots decayed to within 1½ inches of the soil surface. All are putting out lateral roots from the lowermost sound point. Similarly injured seedlings when transplanted lived and made satisfactory growth.

cases. It may furthermore be that in other cases the disinfectant had a stimulating effect, resulting in better germination in the treated plats, entirely aside from that resulting from parasite control. The number of disinfectant methods which concurred in giving apparent increases in germination, however, makes it seem reasonably certain that no great part of the increase was due to this stimulation.

In addition to the different disinfectants shown in the table, mercuric chlorid, heat, hydrochloric acid, nitric acid, and ammonia all apparently resulted in approximately the same increases of germination in tests at the Bessey Nursery as the sulphuric acid which was used as the standard for comparison in most of the series. Relative emergence in treated and untreated plats, as well as damping-off

FIG. 7.—A clean-killed area in a bed of *Pinus ponderosa*, caused by *Corticium vagum*. Inside a 12-inch circle at the center of this "patch" no seedlings appeared. It will be noted that the weeds as well as the pines have been killed with the exception of *Salsola tragus*.

loss after the seedlings appeared, was determined at two nurseries in addition to those given in the table. The results at these nurseries in general confirmed those at the five nurseries covered by the table in showing lower emergence in the controls. Although it is impossible to draw positive conclusions, some idea of the seriousness of losses before the appearance of the seedlings above ground can be obtained by studying the data in Table I. The fact that such losses appear considerable, sometimes exceeding the losses from the damping-off that occurs after emergence, is believed to explain the common failure to secure satisfactory results from control measures taken after the seedlings have come up and the disease has become noticeable. It is somewhat interesting to note that the data in the

table tend to confirm field observations that, as compared with other species, *Pinus resinosa* is more susceptible to the later forms of damping-off than to germination loss.

Further indication that the killing of germinating seed before emergence may be important enough to help explain cases of poor germination is obtained by an entirely different method, as follows: At the Wind River Experiment Station of the United States Forest Service counts of the seedlings emerging and of those which later died were made on a number of untreated plats by forest officers, who kindly permitted the writer to use the data obtained. The counts were made separately on 10 plats each of noble fir (*Abies nobilis*) and silver fir (*Abies concolor*). The plats of each species had been sown with equal quantities of seed. It appeared on inspection of the figures that the plats which showed the poorest emer-

FIG. 8.—The area shown in figure 7 after the bed had been weeded and damping-off had practically ceased. (Photographed by S. C. Bruner.)

gence were also the ones which suffered the most subsequent loss. The coefficient of correlation between the number of seedlings emerging and the percentage of subsequent loss in the same plats was found to be -0.49 ± 0.16 for the noble fir, and -0.50 ± 0.16 for the silver fir, an average of -0.49 ± 0.11 for the two species, confirming the conclusion drawn from inspection of the figures. In other words, poor emergence and heavy subsequent loss were in general associated. The simplest explanation of this association appears to be to suppose that both poor emergence and subsequent loss were largely due to the same cause, namely, the damping-off parasites. Another possible explanation of the correlation would be to neglect parasites as important causes of the poor emergence in certain plats and to suppose that the higher subsequent loss in such plats was due to heat injury, the less dense stands affording less shade to the bases of the seedlings composing it. As damping-off is in general so much more important than heat injury as a cause of death after emergence and the difference in the degrees of shade between the plats with the denser

and the plats with the thinner stands must have been very slight, this latter explanation has not much to support it. The data are believed to constitute further evidence of the importance of parasites in decreasing the percentage of emergence in coniferous seed beds. That the effect of parasites on emergence should have been large enough in this case to make itself apparent on the face of the figures, despite the variations due to other sources, is especially interesting in view of the fact that the losses after emergence in these plats were not high.

ECONOMIC IMPORTANCE OF DAMPING-OFF.

The importance of damping-off in coniferous nurseries in Europe is indicated by frequent reference to the disease in the literature. Büttner (25, 26) states that whole beds are frequently destroyed by it. Baudisch (9) speaks of the death of entire stands in many nurseries as the result of damping-off. In the United States Spaulding (137) considers damping-off a serious obstacle in forestation operations. Clinton (28, p. 348–349) reports serious damage to conifers in New England nurseries. The writer has found the disease especially prevalent in nurseries in Nebraska and Kansas, a somewhat unexpected situation in view of the relatively dry conditions prevailing there. A correspondent has reported heavy loss in seed beds in Texas.

The economic importance of the disease in conifers is due in part to the rather heavy average losses experienced at many nurseries and in part to the irregular character of the losses. In one season losses may be negligible, while the next season the beds of certain species may be practically wiped out. Even without this element of uncertainty the losses experienced are expensive, because of the high cost of coniferous seed. The seed of some species costs from $3 to $5 a pound and seldom shows a germination of more than 60 per cent under nursery conditions. A loss of half of this 60 per cent from parasites, both before and after the seedlings break through the soil, is therefore a matter which deserves attention. The figures in column 8 of Table I, obtained by adding together those in columns 6 and 7, show that the loss is frequently higher than this. At the nurseries at which control experiments have been conducted, the percentage of the seedlings in untreated beds which have been found by actual count damped-off after emergence is frequently more than 50 per cent, in addition to the considerable but less accurately determinable loss indicated by the foregoing data as being caused by the parasites before the seedlings appear.

It has been suggested by foresters and others that the net economic damage from damping-off is not as great as is indicated by the loss of seed and seedlings which it may cause. The argument is ad-

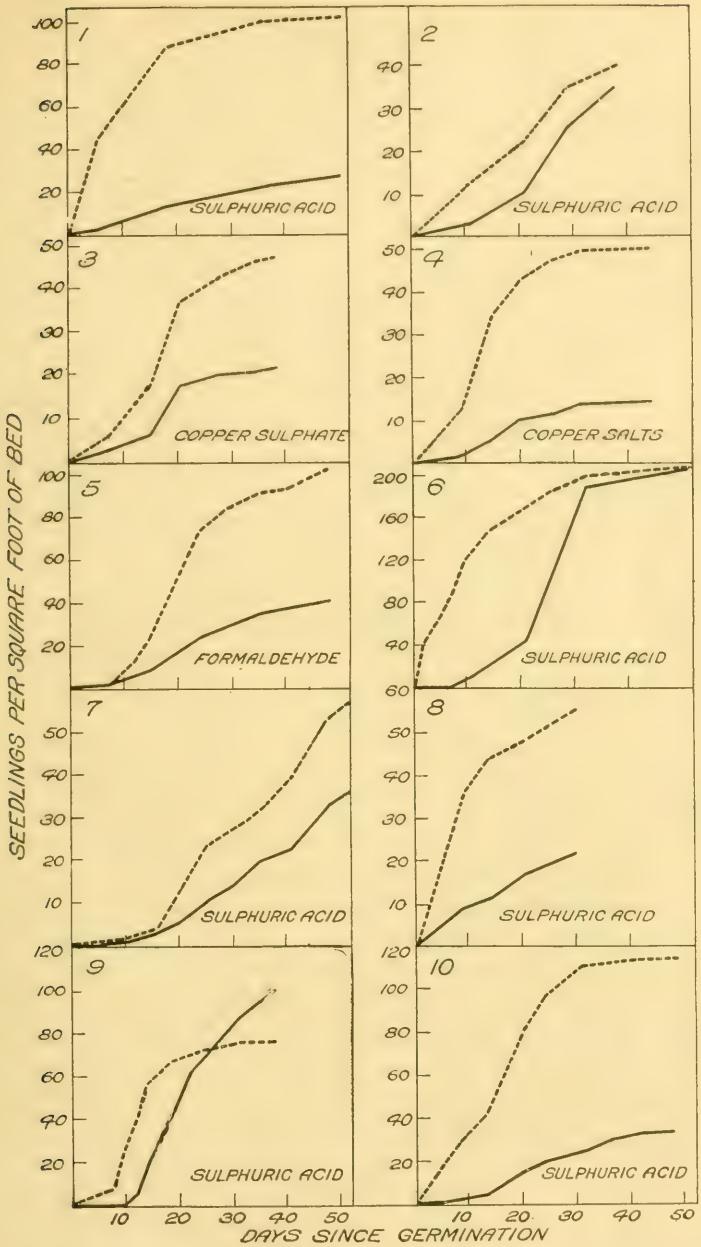

FIG. 9.—Diagram showing the progress of damping-off in treated plats (solid line) as compared with untreated plats (broken line). Graphs 1 to 4 represent *Pinus ponderosa;* graphs 5 to 8, *P. resinosa;* graph 9, *P. banksiana;* and graph 10, plats each of which was half *P. nigra poiretiana* and half *P. sylvestris.* Nurseries in Kansas, Nebraska, Minnesota, and Michigan are represented. The relatively high total number damped-off in the treated plats shown in two of the graphs is due to the fact that a large proportion of the seedlings in the untreated plats had been killed before they appeared above the soil surface. In both the cases in which the absolute number of seedlings killed was as great in the treated plats as in the controls, the percentage of the seedlings killed was nevertheless lower and the survival more than twice as good as in the controls.

vanced that damping-off may be a valuable selective agent in nursery-grown stock for forest planting, eliminating the weaker individuals and thus insuring the vigor of the trees which go into the forest plantation. This is a possibility which must be considered. It is by no means certain, however, that escape from damping-off is correlated with permanently superior vigor. It is believed that temperature, moisture, and other environmental factors, which as yet are very imperfectly analyzed, together with the age of the seedlings and the presence or absence of virulent strains of the parasites, are much more important factors than inherent differences in individual resistance in determining whether or not seedlings are destroyed. Evidence from inoculation experiments and from field observation, supported by data of the sort presented in Table I, indicate that damping-off ordinarily does a considerable part of its damage by killing the sprouting seed before emergence from the soil, while the graphs in figure 9 show that of the loss which occurs after emergence in untreated beds a large part occurs very early in the life of the seedlings. Observation of the clean sweeps which the disease commonly makes in the immediate neighborhood of infection foci (figs. 3, 4, and 5) indicates that either before or just after the seedlings break through the soil none of them have any considerable resistance to the really virulent strains of the parasites, which are believed to be the ones responsible for the major share of the damping-off.

Even if there should be found to be an appreciable selective value in damping-off, this would not be a valid argument against control by seed-bed disinfection for the following reason: The graphs showing the course of damping-off in treated plats in figure 9, together with the decided differences in germination between treated and untreated plats, indicate that the very early damping-off is more completely controlled by disinfectants than the later damping-off. This early damping-off which the treatments so largely prevent is the part of the loss which has the least possibility of selective value. The later damping-off is rarely controlled at all thoroughly by disinfectants. As shown by the graphs, it is often even heavier on the treated than on the untreated soil. It is the part of the loss which is most likely to have selective effect. At this stage beds are not taken clean, as earlier; only seedlings which are below normal resistance succumb. The damping-off in disinfected beds seems therefore at least as likely to have true selective value as that which occurs in untreated beds.

The only way in which the effect of damping-off as a selective agent can be positively determined will be to compare through several subsequent years the growth rate, or survival after transplanting, of trees from beds which suffer seriously from damping-off with the growth of trees from the same lot of seed in seed beds in

which the disease is either accidentally absent or is artificially controlled. Such an experiment is within the silvical rather than the pathological investigative field. If it be found that there is some selective value in the action of the disease and that it is greater in untreated than in treated beds, it would still seem that a much more desirable and dependable selection could be obtained by discarding weak plants at the time of transplanting than by letting damping-off run uncontrolled in the seed beds. Damping-off is sometimes negligible and sometimes destroys practically all the seedlings in a given area, in neither of which cases can it have any material selective value.

RELATIVE SUSCEPTIBILITY OF DIFFERENT CONIFERS.

Büttner (25) writing of European conditions, states that exotic conifers are especially subject to damping-off. He includes fir, spruce, pines, larches, and cypress in this statement. He mentions the same subject in a later paper (26). Neger and Büttner (94) give a long list of different species of conifers from various parts of the world with statements as to their susceptibility to damping-off. Beissner (11, p. 656–657), Neger (93), Clinton (28), Bates and Pierce (7), Boerker (13), and Tillotson (139, p. 69) have all given information on the susceptibility of different conifers. The data reported by Tillotson are drawn from reports by various officers of the United States Forest Service which he has compiled. While it is probable that the nurserymen who are responsible for most of his records have not observed the disease as closely as Neger and Büttner, the fact that their observations are mostly based on repeated seasons' work with large-scale seed beds of the species they report on makes their observations in some respects more reliable than the other published data. Neger and Büttner presumably worked in most cases with small beds of the various conifers on which they report, and the variations which they attribute to differences in specific resistance might easily in such case be largely due to accidental variation. The error which nurserymen are most likely to make in their notes on susceptibility is to underestimate the loss, especially for the small-seeded species. The seedlings of small-seeded conifers decay and shrivel so quickly after they fall that in taking notes at any one time only a small proportion of the total loss is visible. Frequent counts of dead seedlings are the only way by which the loss after germination in such species can be properly appreciated.

The data given by the authors mentioned in the foregoing paragraph, together with unpublished data obtained by personal observation or from commercial and other nurserymen in the United States, are summarized in Table II, the source of each report being shown by letters signifying the authority. The unpublished data on two

nurseries in Illinois and Minnesota were obtained from the nurserymen by Mr. R. G. Pierce and are indicated by the initial "P." Information obtained directly from the nurserymen by the writer is indicated by "N." For nurseries where the statements are based on the writer's personal observation rather than on the authority of the nurserymen, his own initial ("H") is given. Most of the writer's own estimates of relative susceptibility are based on a comparison of detailed counts of the damped-off seedlings in a large number of untreated plats at different times, as well as on observation. The nurseries on which Tillotson reported were all west of the Missouri River, most of them being in the mountain region. The reports indicated by "H" and "N" were mostly from nurseries east of the Rocky Mountains. In cases in which the data permit it, the species are classified as most susceptible, intermediate, least susceptible, or immune. In a number of cases, however, it is only possible to classify them as "more" or "less" susceptible.

TABLE II.—*Relative susceptibility to damping-off of different conifer species.*

[Figures in parentheses in this table indicate the number of nurseries from which the susceptibility noted has been reported by the observer to whom the preceding letter refers.]

Host species.[a]	Reports of relative susceptibility.[b]					
	Not susceptible.	Least susceptible.	Less than average.	Intermediate.	More susceptible than the average.	Most susceptible.
Pinaceæ (Abietoideæ):						
Abies spp		(c)				
Abies balsamea		Nb				
Abies cephalonica				Nb		
Abies concolor						Bu, Nb.
Abies nobilis			T			Nb.
Abies nordmanniana				Nb		
Abies picea (A. pectinata)				Nb		
Abies sacchalinensis (?)				Nb		
Abies sibirica		Nb				
Abies veitchii						Nb.
Cedrus deodara			T			
Larix europæa				Nb	N (2)	
Larix leptolepis						Nb.
Larix occidentalis			T		T	
Picea ajanensis			Ne	Nb		
Picea canadensis		Nb		P	N	
Picea engelmanni			T (2)		H, N, Ne, T.	Nb.
Picea excelsa	P	Nb	H, Bu		Ne, N	
Picea omorika			Ne	Nb		
Picea orientalis			Ne	Nb		
Picea pungens			N (2)	H, P	Ne	N (2), Nb.
Picea sitchensis					Ne	Nb.
Pinus aristata		Nb				
Pinus attenuata					N	
Pinus banksiana		Nb	N	P	B, P, H (2), T.	B., H.
Pinus contorta	T	Nb	T			
Pinus edulis	T					

[a] Host names for American species follow the usage in the publications and a later verbal communication of Mr. George B. Sudworth, of the United States Forest Service. For exotic species the Standard Cyclopedia of Horticulture, New York, 1916, edited by L. H. Bailey, is taken as the standard. The classification follows Saxton (118).

[b] Symbols signifying the authority for the report: B=Boerker (13), Bu=Büttner (25, 26), Bp=Bates and Pierce (7), C=Clinton (28), H=Writer's estimate, N=Nurserymen's estimate (obtained by the writer), Nb=Neger and Büttner (94), Ne=Neger (93), P=Nurserymen's estimate (obtained by Pierce), T=Forest officers' estimate (compiled by Tillotson, 139).

[c] Susceptibility to *Phytophthora fagi*.

TABLE II.—*Relative susceptibility to damping-off of different conifer species*—Continued.

Host species.	Reports of relative susceptibility.					
	Not susceptible.	Least susceptible.	Less than average.	Intermediate.	More susceptible than the average.	Most susceptible.
Pinaceæ (Abietoideæ)—Continued.						
Pinus excelsa		Nb.				
Pinus flexilis		N				
Pinus jeffreyi			T		T (2)	
Pinus lambertiana					T	
Pinus montana mughus				P		
Pinus monticola	T		T		T	
Pinus nigra austriaca (Austrian pine)		Nb	N, T (3)		Bp, N, T	
Pinus nigra poiretiana (Corsican pine)	Bp			H		
Pinus palustris				B		
Pinus peuce		Nb				
Pinus ponderosa (type not specified)	T (3)	P	Bp, N (4), T (6).	Nb	T (4)	
Pinus ponderosa (Eastern Rocky Mountain type)			H (1)		B	
Pinus ponderosa (Pacific coast type)			B			
Pinus resinosa			N (3)		Bp, H, T	B, H.
Pinus rigida			P		N	
Pinus strobus	P		B, H, N	N	C, N, T	
Pinus sylvestris			T (2)	H, P	Bp, T (3)	N.
Pinus taeda		B				
Pinus thunbergii		Nb				
Pseudotsuga taxifolia (type not specified)			N (3) T (3)	H	N, T (5)	
Pseudotsuga taxifolia (Colorado type)		Nb			B	
Pseudotsuga taxifolia (Northwestern type)		B		Nb		
Tsuga canadensis				Nb, P	Bu	
Number of reports	9	17	51	23	48	15
Percentage of total	6	10	31	14	29	9
Sciadopitoideæ:						
Sciadopitys verticillata						Nb.
Cupressaceæ (Cupressoideæ):						
Chamaecyparis lawsoniana		Nb	T		Bu	
Chamaecyparis pisifera		Nb				
Cryptomeria japonica				Nb		
Cupressus spp		Nb				
Cupressus arizonica	N					
Juniperus communis	Nb			P		
Juniperus monosperma	N					
Juniperus pachyphloea	T					
Juniperus virginiana	H, P	Nb				
Libocedrus decurrens	T	Nb	T			
Taxodium distichum		Nb				
Thuja occidentalis	P	Nb				
Thuja orientalis	P			Nb		
Thuja plicata				Nb		
Number of reports	9	7	2	4	1	0
Percentage of total	39	30	9	17	4	0
Sequoideæ:						
Sequoia spp					B	
Sequoia washingtoniana					T	
Taxaceæ:						
Taxus cuspidata	P					

The fact most evident in Table II is the extreme variation between reports, not only on closely related species but even on the same species. While it is, of course, possible that the obvious lack of a definite basis and method of comparison in most of the reports is responsible for most of this variation, it seems to the writer more probable that different species do actually vary in their relative susceptibility to damping-off in different localities. In the first place,

the conditions which might increase resistance of one host might very easily decrease its resistance for a host with different environmental requirements. To illustrate by an extreme example, the piñon (*Pinus edulis*) of the arid or semiarid region might remain resistant in soils in which *Picea engelmanni* of the high mountain-stream bottoms or *Picea mariana* of the northern swamps might be low in vigor and easily attacked. In the second place, it is to be expected that species with a certain order of relative susceptibility to the parasites which predominate at one nursery may exhibit a very different order of susceptibility to the different combination of parasites which might be prevalent in another locality.

The only individual species on which there are a sufficient number of reports and a sufficient agreement between the reports are the two common western spruces, *Picea pungens* and *P. engelmanni*, which (at least as compared with *Picea excelsa*) seem rather susceptible, and *Pinus ponderosa*, which (as compared with most of the other species of the Abietoideæ) is to be regarded as generally more resistant than the average. Within each of the larger genera of this group it seems evident that susceptibility is extremely varied and that no statement as to the relative susceptibility of the genera themselves can therefore be made. The only group generalization that is perhaps permissible is derived from the consideration of the Cupressoideæ. In this group, out of 23 reports, 16 are in the "not susceptible" or "least susceptible" columns and only one indicates more than intermediate susceptibility. Of 163 reports pertaining to the Abietoideæ, only 26 place them in the "not susceptible" or "least susceptible" columns and 63 in the classes of more than intermediate susceptibility. The general feeling among nurserymen seems to be that serious damping-off need not be feared among the cedars and their relatives. The data at hand tend to justify this confidence.

CONTROL OF DAMPING-OFF.

Early efforts to prevent damping-off were chiefly directed to the avoidance of excessive moisture in either the air or soil. A means to this end, which has been observed more or less by nurserymen for many years, both in the United States and elsewhere, is the application of small quantities of dry sand to the seed beds after the disease becomes noticeable (18, p. 166; 83). This is sometimes applied hot (101, p. 43–44; 145), though even this procedure does not result in very great advantage. Surfacing with hot sand can not always be counted on to give any measurable advantage over untreated beds (67, p. 3). The use of sand (25) or sterile subsoil (101) instead of ordinary soil in covering seed at the time of sowing has been advised. Johnson (82) did not secure satisfactory results with sand in tobacco

beds. Making the upper part of the bed to a depth of several inches of recently dug subsoil appeared effective in a single test by Spaulding (137) and at four nurseries by cooperators of the writer in later tests, the results of which will be published elsewhere. The procedure is unfortunately rather expensive in large-scale work and under some conditions at least undesirable because of the poor subsequent growth on such soil. Excessive vegetable matter (45), imperfectly rotted manure (67), or green manures recently plowed under (43) have all been advised against as likely to favor the disease. The experience reported with conifers (67, 139) indicates that damping-off can be to a certain extent decreased by broadcast sowing as compared with sowing in drills. The usual recommendation of thin sowing to avoid the seed-bed disease of other plants has also been made for conifers (67). Transplanting healthy seedlings from infected beds into new soil is recommended as a means of saving them from attack (11, 145). The writer's tests of transplanting at a Nebraska nursery gave no promise of economic value as a control method, although he is informed that it was successfully employed in a nursery in New Mexico. The time of sowing appears to have a relation to the amount of disease at some nurseries, but conditions in this regard evidently differ in different localities, so that the best time to sow from the standpoint of avoiding damping-off must be determined separately by repeated tests at each nursery. For example, observations both by the nurserymen and the writer during several seasons at the Bessey Nursery, in Nebraska, indicate that fall sowing is an excellent means of decreasing loss from damping-off in at least one pine species, and Retan (110) reports the same thing for a nursery in Pennsylvania, while at two Kansas nurseries and at nurseries mentioned by Tillotson (139) fall-sown beds suffer more than those sown in the spring.

Treatment of the seed with mercuric chlorid (25) or with copper sulphate (122) has been recommended. While it has been demonstrated (38) that a proper heat treatment of the seed will greatly decrease the damping-off in sugar-beet seedlings, this is explained by the fact that one of the most important parasites of the sugar beet is systemic and often present in the seed. There is no reason to believe that seed-carried infection is of any importance in coniferous seed beds. The only advantage that could reasonably be expected from a seed treatment of conifers would be that which would come from the prevention of seed decay in the soil before germination starts, and this protection could be expected to be effective only if a relatively insoluble disinfectant, such as Bordeaux mixture, was used.

Soil treatment is the most direct and probably the most profitable method of attack on the disease. It is especially easy, for tobacco

seedlings (82) as well as for pines, to prevent by soil disinfection losses before the seedlings appear above the ground. Heat disinfection of seed beds has been frequently mentioned. Burning wood or litter on the surface of the beds before sowing, said by Gilbert (47, p. 36) to be a common procedure in preparing tobacco seed beds both in Italy and in parts of this country, has been recommended for coniferous seed beds by Büttner (25). The disadvantageous results sometimes noticed following the application of wood ashes to pine seed beds may prove an objection to this type of treatment in some of the nurseries. At a Nebraska nursery (67) moist heat proved only partly satisfactory, unavoidable reinfection having serious results. Steam disinfection, using the inverted-pan method commonly advocated for tobacco seedlings (10, 47, 81), has been reported by Scott (123) as successful at a nursery in Kansas. Gifford (46) found steaming with the inverted pan only partly satisfactory. It is not believed that it is likely to pay to install the necessary apparatus for steam disinfection at nurseries in nonagricultural districts where steam tractors are not available for temporary use. The hot-water soil treatment as used by Byars and Gilbert (27) is probably worth a trial at any nursery where damping-off is serious and fuel cheap. It may be that in some localities where steam or hot-water treatment of the soil is not sufficiently effective, its efficiency can be increased by reinoculating the soil immediately after treatment with saprophytic molds and bacteria to provide maximum competition for parasites which come in from the outside. Tests of this procedure will be described later in the present bulletin. The value of charcoal has been emphasized by Retan (109, 110).

Chemical disinfection of the soil has also been employed. Sulphur has long been in use as a soil treatment against the damping-off of various plants (45, 111) in addition to its use in combating potato scab and onion smut. It was tested on conifers by Spaulding (136, 137) in the form of light surface applications to the beds after germination, but without decisive result. In later cooperative tests powdered sulphur raked into the soil before the sowing of the seed failed to indicate any large measure of value. Very finely divided forms of sulphur in various amounts and times of application are probably worth some further test.

Möller (90) and Sherbakoff (128) have reported the successful use of copper sulphate in combating attacks of Corticium on dicotyledons. In Johnson's experiments on tobacco seedlings (82, table 3) copper salts and Bordeaux mixture were the only chemicals for which any value was indicated. Sherbakoff apparently used copper sulphate and other strong disinfectants chiefly to stop the extension of vigorously spreading damping-off foci by local treatment rather than as a general treatment for use over the beds. Such treatment

would presumably kill all seedlings on the area treated, but would, of course, be of considerable value in stopping at the outset such mycelia as those which caused the damped-off area in figure 7. The procedure would be of practical value only in cases in which damping-off was chiefly limited to a few large patches of this sort, a rather rare condition in conifers.

Copper sulphate solutions have been used on pine seed beds at the time of sowing with considerable success at some nurseries (65, 67). Except in a nursery in which the soil contained carbonates, it has proved rather difficult to prevent injury to the pines. The trial of some such combination of copper sulphate and lime as was used by Spaulding (136) on the surface of pine beds before sowing, which apparently prevented the damping-off of lettuce in some unpublished pot experiments of Mr. J. F. Breazeale, is considered desirable. Treating seed beds with ordinary Bordeaux mixture has also been recommended. Horne (78) secured especially good results against *Corticium vagum* in tobacco seed beds by heavy applications of Bordeaux mixture, and Schramm (122) and Clinton (28) have obtained indications of its value as a spray in preventing the damping-off of conifers. It is probably worth further tests in various amounts of application. In tests conducted by the writer in 1912 and still unpublished, some advantage was indicated for Bordeaux mixture as a surface treatment after soil disinfection with acid. Zinc chlorid as a soil disinfectant has also been found valuable in a number of cases (65, 67), but it is more expensive and apparently less dependable than copper sulphate.

Formaldehyde and sulphuric acid have been tested more frequently than other disinfectants. The use of sulphuric acid on coniferous seed beds was originated by Spaulding (136). The first intensive experiments with this acid were reported by the writer (63). The first experiments with formaldehyde on conifers seem to have been in the early greenhouse tests of Spaulding (137), repeated in forest nurseries in 1907 by Jones (83) and Spaulding (136). Most of the experiments with these two substances have already been summarized (67). A report not mentioned in this summary is that of Schaaf (119, p. 88), who obtained favorable results with the acid. The great trouble with formaldehyde is its tendency to kill dormant seed. The length of time which must be allowed to elapse between treatment and sowing in order to avoid this killing varies with conditions. Formaldehyde is more expensive than acid and seems on the whole to have been less effective in disease control. Acid, on the other hand (applied just after the seed is sown, which is found to be the best time), on a few soils has caused injury to radicles, which it was at first thought could be prevented only by very frequent watering during the germination period; while in a few cases, when cold

weather resulted in a long germination period, it has killed or inhibited the germination of some of the dormant seed. All injury can be prevented by treatment a few days before sowing, followed by the addition of lime just before sowing, but lime used in this way has apparently destroyed a considerable part of the value of the acid treatment against the disease. Further consideration of the data on which earlier papers (63, 67) were based indicates that the apparent need for frequent watering during the germination period, which was required at a few of the nurseries where the first tests of acid treatment were made, as well as practically all of the germination reduction, was due to the use of unnecessarily large applications of acid and that the trouble can be eliminated by determining by test the minimum quantity of acid which will be reasonably effective in each locality. If this can be done it should establish the acid treatment as the most profitable for general use of any of the methods of damping-off control which have so far been extensively tested.

In view of the various parasites which may cause damping-off at different times and places and which vary greatly both in their means of dissemination and in their physiological qualities, it is not believed that any single disinfectant will be found entirely satisfactory at all nurseries. It is also unfortunately true that no one strength of treatment can be recommended for all nurseries. The quantity of acid to be used at any specified nursery will have to be determined by test at that nursery. A single test, no matter how well conducted, is not sufficient to serve as a basis for conclusions. However, a number of small-scale tests, made at different times and in different parts of the seed-bed area, should determine the best treatment for any particular nursery with a reasonable degree of certainty and with very little work. If the plats are equal in size and receive equal quantities of seed, all the nurseryman needs to do to determine the value of the treatments is to count the number of living seedlings on the different plats at the end of the season. The decrease in the number of weeds as a result of the use of acid is itself sufficient at a number of nurseries to pay the entire cost of the treatment. Detailed methods of application have already been published (67). The differing proportions of acid between which the best treatment will ordinarily be found to lie are 2 and 7 c. c. (one-sixteenth and one-fourth of a fluid ounce) of the concentrated commercial acid per square foot of seed bed, applied just after the seed is sown and covered. It should be dissolved in 500 to 1,000 c. c. (1 to 2 pints) of water per square foot of bed before applying. The drier the soil before treatment, the more water should be used in dissolving the acid.

No treatment applied after germination begins can have the maximum value in controlling the disease, because the damping-off para-

sites frequently, if not usually, do part of their work before the seedlings appear above the soil. Furthermore, any treatment at all effective against the disease is almost certain to hurt the seedlings if applied after the seed starts to sprout.

Both the acid and copper-sulphate treatments which have been found useful in pine seed beds are of very doubtful value for most hosts other than conifers, as the angiosperms on which observations have so far been made are too easily injured by the disinfectants. The weeds in the nurseries have been injured or entirely kept from appearing by treatments which caused no injury to the pines.

CAUSAL FUNGI.

CORTICIUM VAGUM.

Occurrence and parasitism.—In a recent publication (68) *Corticium vagum* B. and C. (*C. vagum solani* Burt, *Hypochnus solani* Pril. et Del., the common damping-off Rhizoctonia) has been reported on a number of conifers. Inoculation, reisolation, and reinoculation on pine have established its parisitism on this host beyond a reasonable doubt, though in these inoculations, as in most, if not all, the work which has been done with the fungus on angiospermous hosts, the cultures employed have been from plantings of diseased tissue instead of from single spores. The inoculation experiments have confirmed the field observations indicating that this fungus is fully as able to cause loss by destroying germinating seed below the soil surface as to cause damping-off of the better known type after the seedlings appear above the soil surface.

An extensive list of angiosperms on which the fungus has been reported is given by Peltier (98). Cross-inoculations between the pines (68), on the one hand, and potato (40) and sugar beet (38) have shown the same strains to be parasitic on both conifers and angiosperms and established the physiological as well as the morphological identity of the fungus attacking pines with the common *Corticium vagum*. Now that Duggar (34) has offered strong, though not yet entirely conclusive, evidence of the identity of *C. vagum* with the European "vermehrungspilz" (the *Moniliopsis aderholdii* of Ruhland; 115) it is to be presumed that it is a cause of damping-off of conifers in Europe as well as in America, though no reports of it on conifers have been so far encountered in European literature. The Rhizoctonia reported by Somerville (132) on *Pinus sylvestris* and the *Rhizoctonia strobi* described by Scholz (121) as killing young *Pinus strobus* were both on trees more than 4 years old, so that they had no relation to damping-off. Furthermore, the first of these was apparently the old *Rhizoctonia violacea*, now known as *R. crocorum* (*R. medicaginis*), a fungus entirely distinct from *Corticium vagum*, probably belong-

ing to an altogether different group of fungi and not known to cause damping-off of any host. *Rhizoctonia strobi* is not sufficiently described to allow determination of its identity.

Variations in virulence.—In the inoculations earlier reported on conifers, different strains of *Corticium vagum* were said to vary greatly in virulence (68). Further examination of the data on which this statement was based yields confirmatory evidence. Part of this evidence is shown graphically in figures 1, 2, and 10. The experiments on which these graphs were based involved at the time of seed sowing the addition to the soil of apparently pure cultures of *C. vagum*. Throughout each experiment the different units received

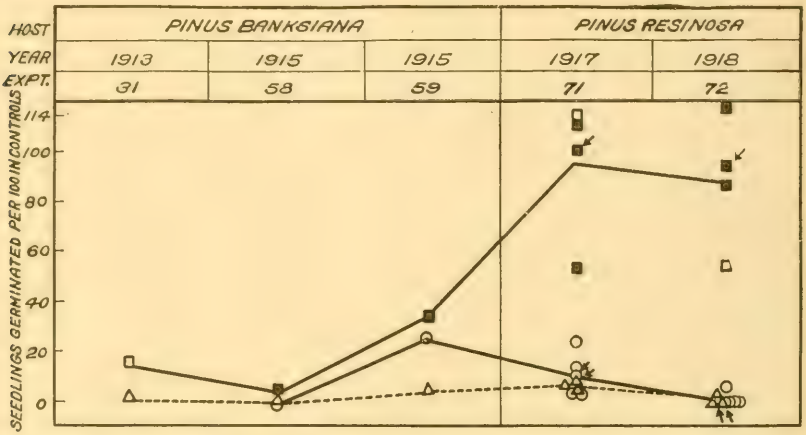

FIG. 10.—Diagram showing the relative activity of different strains of *Corticium vagum*, as indicated by the number of seedlings appearing in inoculated pots. Explanation of symbols: ○ =Strain 147, from spruce seedlings, Washington, D. C., 1910; ▽ =strain 213, from sugar beet seedlings, Washington, D. C., 1911; ▣ =strain 230, from *Elaeagnus* sp., Kansas, 1913; □ =strain 233, from the same lesion as strain 230. Strains reisolated from these, the results of which appear in experiments Nos. 71 and 72, are indicated by the same signs as the original strains used in the inoculations from which they were taken. The original strains in experiments Nos. 71 and 72 are indicated by arrows.

equal quantities of seed, and the culture substratum used in inoculating was the same for all strains. Experiments 36, 45, 47, 49, and 51 were conducted on plats in out-of-door drill-sown beds, experiment 36 on an alkaline soil, all of which had been heated in a moist condition at a temperature of not less than 80° C. for not less than 10 minutes,[2] and experiments 45, 47, 49, and 51 on a sand which had

[2] This temperature is probably high enough to eliminate damping-off organisms. Tests by Dr. Theodore C. Merrill indicate that the three most virulent parasites so far worked with are killed by placing agar tube cultures for 10-minute periods in water at the following temperatures: *Pythium debaryanum*, 65° C.; *Corticium vagum*, 50° C. for mycelium and 60° C. for sclerotia; *Fusarium moniliforme*, 70° C. Both the Pythium and Fusarium cultures contained spores. The possibility of the survival of oospores which would not be capable of germination for several months was apparently eliminated by the writer, who retained Dr. Merrill's heated Pythium tubes and made final transfers from them 7½ months after heating, still without securing growth. Plenty of typical oospores were present in the part of the heated culture from which transfers were made.

been treated with sulphuric acid followed later by lime. The other experiments included in the graphs were on autoclaved sandy loams in pots in the greenhouse. In these graphs are included all of the results in which the same groups of strains were used repeatedly in different experiments. In figure 1, the values plotted for experiments 36, 49, and 51 are for the number of seedlings which appeared above ground, the heavy inoculations and favorable conditions for damping-off in these experiments being such that even weak strains caused heavy losses and the survivals therefore do not give differential results. Comparison of the survival data in the other experiments in figure 1 with the emergence data for the same strains in that figure and in figure 10 indicates that the strains best able to reduce survival are also the ones best able to reduce emergence.

While the data presented in the graphs are not entirely consistent, it is very evident from them that strains 147, 213, and in a lesser degree 206 were regularly more virulent than most of the strains in tests conducted several years apart on different species of Pinus. It is also evident that certain strains of 186 and 189 which appear in figure 2 are quite regularly of low or doubtful virulence. Strains 50, 183, 192, 211, 212, 230, and 233, whose virulence is apparently intermediate, show a greater variability. In experiments 36, 45, 47, 49, and 51, in which conditions especially favor parasitism, they may cause practically as serious loss as the regularly virulent strains, the best differential results being shown in experiments in which the disease is less favored. The apparent variation in the relative virulence of such strains in different experiments may, of course, mean that their virulence is differently affected by different conditions. It seems rather more probable that the variation in relative activity is to be classed as accidental variation, necessarily great with small units which are subject to numerous uncontrollable variables. It seems entirely possible, however, that part of the observed differences in relative activity may be due to differences, not in virulence, but in the ability of the different strains to maintain themselves saprophytically in different soils during the period between inoculation and the commencement of germination. For example, strains 230 and 233 came from a nursery in southwestern Kansas in which the soil-acidity exponent, as determined by Dr. L. J. Gillespie, of the United States Bureau of Plant Industry, is 8.4. It seems entirely possible that these strains, rather strongly parasitic in some of the experiments, including an experiment on the soil from which they were taken, might prove less able than strains from some other habitats to maintain themselves on some of the eastern soils used in the greenhouse tests. The source of strains 230 and 233 was furthermore a locality where high soil temperatures are to be expected. The fact

that experiments 71 and 72, in which they showed the least virulence, were conducted in a colder greenhouse than any of the other tests may have had something to do with the lower activity indicated for these strains. Variation in the temperature requirements of different strains in accordance with the temperature of the source locality has already been demonstrated by Edgerton (35) for one of the anthracnoses. It is hoped later to determine the temperature and acidity preferences of these two strains as compared with the others used. It should be noted that the consistently weak strain No. 189 (fig. 2), was abnormal in habit, lighter brown, and produced fewer sclerotia than typical strains. The other strains appearing in the graphs showed no conspicuous morphological or cultural differences that were identical. The only other strain which was noticeably abnormal in culture was one from pine seedlings in Kansas, intermediate in habit and color between No. 189 and the typical strains and indicating little more virulence than No. 189 in the few experiments in which it was used. It does not appear in figures 1 and 2, but was included in the experiments reported in the following paragraph. Peltier (99) believes low sclerotium-forming capacity to be a sign of degeneration and low virulence; the writer's experience agrees with his as to virulence, but these two strains showed no other evidence of lack of vigor.

As a further check on the reality of the apparent differences in virulence between different strains, all of the original strains available at the time, a total of 29, were used in the practically duplicate experiments 71 and 72 and the relative survivals of the same strains in the two series mathematically and graphically compared (fig. 11). The very decided correlation between the two experiments indicated by the graph has a coefficient[3] of 0.813 ± 0.042, nineteen times its

[3] The correlation coefficient, a very useful thing for many kinds of biological work, which unfortunately has received little attention from plant pathologists, is explained by Secrist (124, p. 43 et seq.) and the process of computation described (124, p. 453–467). A shorter method of computation is given by E. Davenport (30, p. 465–467); the example he gives is of a series with a large number of varieties, in which the correlation table is employed. Davenport's method is, however, just as useful in such a case as this, in which the number of varieties is too small to make the formal table advantageous. In such a case the computation should be arranged as by Secrist (124, p. 460–461), but the guessed rather than the true mean used and Davenport's formula employed. If the coefficient is $+1$, the correlation is perfect; if it is 0 there is no correlation, and if -1, perfect negative correlation. The significance of a coefficient less than 1 is judged from its relation to its probable error. King (84), in an excellent discussion of correlation, gives rules for judging the degree of significance of the coefficient. The correlation coefficient has its greatest potential usefulness in examining apparent causal relations. It is so used in connection with the relation between the hydrogen-ion exponent and damping-off in considering figure 12 of the present bulletin. Interexperimental, or, as Harris calls them, "interannual" correlation coefficients of the sort used for these Corticium strains have been used by Norton (96, p. 51) in measuring the constancy of rust resistance of asparagus strains, by Harris (54) in demonstrating the constancy of differences in various characters between strains or individuals, and they appear to be useful for this purpose in the present case.

probable error. Peltier's results permit similar correlations for the 18 strains common to his experiments 1 and 1A on carnation cuttings and for the 22 strains common to experiment 1 on cuttings and experiment 2 on seedlings. The coefficients found are decidedly lower than those obtained from the experiments on pine, 0.51 ± 0.117 for the experiments on cuttings and 0.36 ± 0.124 for the comparison of the results on cuttings with the results on seedlings, but nevertheless indicate some interexperimental correlation for the same strains and therefore inherent differences in parasitic ability between the different strains.

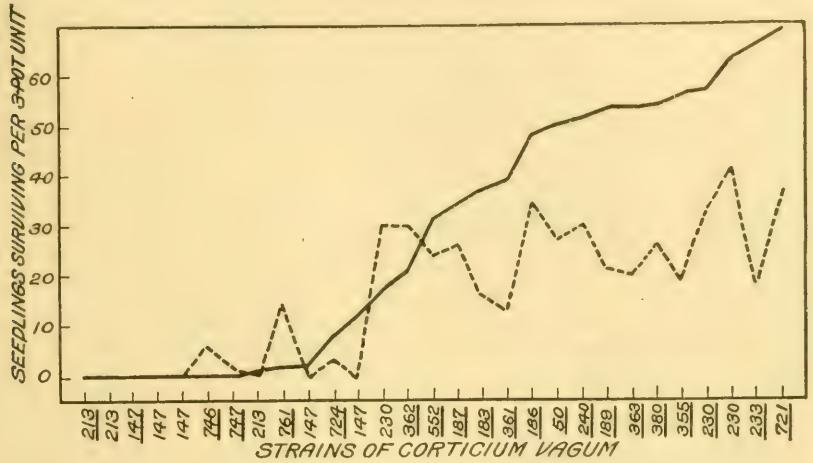

FIG. 11.—Diagram showing the comparative virulence of 29 strains of *Corticium vagum* in two successive inoculation experiments on *Pinus resinosa*. The results in experiment No. 71 are shown by the solid line, the strains being arranged from left to right in the order of descending virulence indicated by the number of seedlings surviving in this experiment. The results from the use of the same strains in experiment No. 72 are shown by the broken line. The obvious correlation between the two curves (coefficient 0.81 ± 0.04) indicates a real difference in virulence between the different strains. The strains indicated by the underscored numbers are original strains and those not underscored reisolations from the original strains in earlier inoculation experiments on pine seedlings.

In the work on pine seedlings, with the possible exception of strains 230 and 233, there was no evidence of attenuation in artificial culture. Strains 147 and 213, isolated in 1910 and 1911, respectively, seemed as strongly parasitic in experiments 71 and 72 (1917 and 1918) as any of the five strains isolated in 1916 or the six strains isolated in 1915.

Of the 20 strains above mentioned, three pairs were isolated under such conditions and showed such later agreement in performance as to indicate their individual identity. For the purposes of consideration in the following paragraph, the one of these probably duplicate strains which happens to have the higher number was eliminated from each pair. The survival figures in pots inoculated with the 17 strains

remaining, giving the mean of the results in experiments 71 and 72, are shown graphically in figure 13, together with the results of some of Peltier's experiments in which other strains were used. Percentages of seedlings damped-off after germination are not included in these and most of the other data on pines because the most virulent strains often entirely prevent germination, and no value for subsequent loss is obtainable. The grouping of most of the writer's strains at the least virulent end of the register (that is, the one with the highest number of living seedlings) is of some interest. The distributions based on the two experiments considered separately

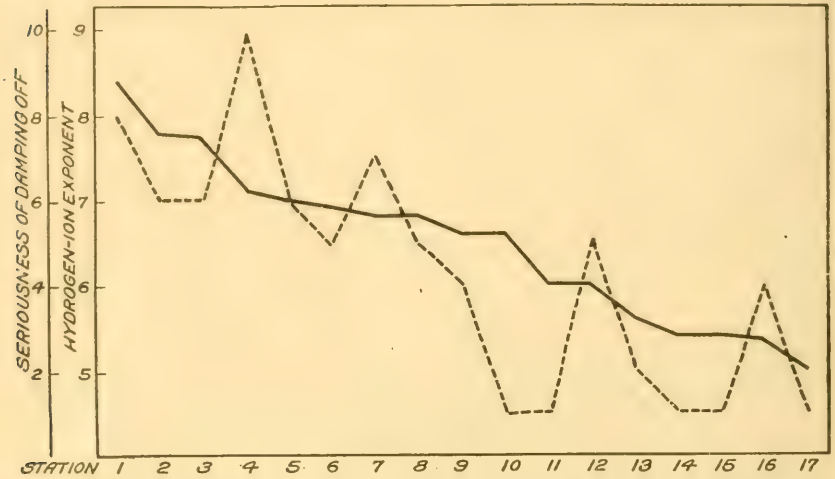

FIG. 12.—Diagram showing the relation between damping-off of conifers (broken line) and soil acidity (solid line). The acidity of soil samples from the different nurseries, determined by Dr. L. J. Gillespie, is reported as P_H7, indicating approximate neutrality while P_H6 indicates ten, and P_H5 one hundred times as great a hydrogen-ion concentration as P_H7; therefore the lower the hydrogen-ion exponent line, the greater the acidity. The seriousness of damping-off at each nursery is on an arbitrary scale in which nurseries with negligible loss are rated as 1, and the nursery which suffered most is rated as 10. These values are estimates, though for some of the nurseries extensive counts were available on which the estimates were based.

agreed very well in this grouping. The minor group at the end of extreme virulence is not taken to indicate an actual grouping but, rather, an artificial one, due to the fact that both the strongest strains and some less strong were thrown into the same group by the lack of additional seedlings for the stronger strains to kill. This lack of additional seedlings constituted a limiting factor. In other words, conditions favored damping-off even in these two experiments too much to permit completely differential results for the more virulent strains. Despite this artificial limit preventing the full variability becoming evident, the coefficient of variability of the survivals

had the high value of 63±9.7 per cent. The graph indicates also a decided, though less extreme, degree of variability for Peltier's strains on carnations; the survivals for the 18 strains which he used in both of his experiments on cuttings have a variability coefficient of 29±3.5 per cent and the 23 strains in experiment 2 on seedlings 55±6.9 per cent.

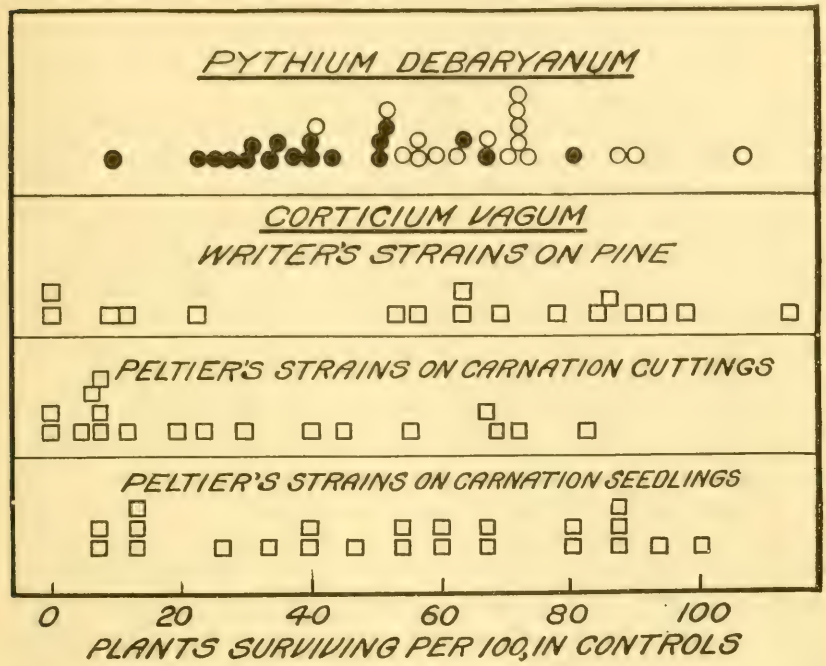

FIG. 13.—Diagram showing the results of inoculations with 17 strains of *Corticium vagum* and 35 strains of *Pythium debaryanum*, arranged in decreasing order of virulence from left to right, as indicated by the survivals in pots of pine seedlings artificially inoculated with them. The Pythium results represent the mean survivals in 5 pots inoculated with each strain in each of experiments Nos. 66, 67, and 68. Each point located is therefore based on the results in 15 pots, 10 of *Pinus banksiana* and 5 of *P. resinosa*. The Corticium results on pine represent 5 or 6 pots each, in two experiments (Nos. 71 and 72) on *P. resinosa*. The outline circles represent *P. debaryanum* strains from East Tawas, Mich.; the solid circles represent strains from other localities. The second row of squares shows the sum of the results in Peltier's experiments Nos. 1 and 1a (99, his table 3). The lowest row of squares shows his results in experiment No. 2 (his table 4).

The strains represented in figure 13, as used on pine, include 1 from bean, 2 from potato, 1 from sugar beet, 1 from Elaeagnus, 2 from *Picea engelmanni*, and 10 from *Pinus resinosa*, *P. ponderosa*, and *P. banksiana*. Two were from Washington, D. C., 2 from New York, 1 from Ohio, 4 from Michigan, 4 from Minnesota, 1 from Nebraska, 2 from Kansas, and 1 from California. The sources of these strains are widely distributed both as to host and locality; they are

rather more representative of the country as a whole geographically than the strains in the larger of Peltier's experiments, though less representative as to host sources. The number is too small to justify conclusions as to the proportion of *Corticium vagum* strains which can be expected to prove strongly virulent on pine. The data are offered merely as a beginning, to which it is hoped experimenters with other strains of *C. vagum* will make additions.

In addition to the strains used in these two experiments, several others which had been lost or for other reasons could not be included in both the final experiments had been previously tested on pines in earlier experiments. Of these, 6 strains, 1 each from alfalfa, sugar beet, *Pseudotsuga taxifolia*, *Pinus banksiana*, *P. resinosa*, and *P. strobus*, gave indications of low virulence on the pines; 3 strains, 1 each from sugar beet, *Pinus sylvestris*, and *P. ponderosa*, gave indications of rather high virulence; while another strain from *P. ponderosa* indicated an intermediate ability to attack pine. Combining these strains with those represented in figure 13, there are data on 27 original strains, of which 8 are roughly classed as strongly virulent on pine seedlings, 14 as weak, and 5 as intermediate.

Edson and Shapovalov (40) have conducted inoculation experiments on potato stems with 6 of the Corticium strains which had been used on pine, including the 5 strains mentioned by them on page 218 and their strains R. XV (the writer's strain 192 of fig. 2) on page 215. Strains 147 and 724, which had proved the most destructive in the inoculations on pine, appeared also rather strongly virulent on potato. Strain No. 186, originally from potato, which had given no definite evidence of parasitism on pine, also proved unable to cause lesions on the potato stems. The remaining 3 strains, all of intermediate virulence on pine, gave results on potato which were less indicative of agreement with the order of virulence on pine. The data suggest that strains strongly parasitic on potato are likely to be strongly parasitic on pine, and vice versa, but the agreement between their results and the writer's is not sufficiently complete to establish the point.

FUSARIUM SPP.

Fusarium is often found on or in damped-off seedlings (24, 46, 60, 94, 120, 137, 141, 142). The early inoculation experiments, conducted in the main with strains not sufficiently described to allow their identification, have been recently summarized (68, p. 537), together with descriptions of inoculation experiments on pine seedlings with four commonly recognized species of Fusarium. These, though not followed by reisolation, gave rather definite evidence that *Fusarium moniliforme* Sheldon was decidedly parasitic and *F. solani* less strongly so. *Fusarium centricosum* Appel and Wollenw. was indi-

cated as more strongly parasitic than *F. solani*, but in a single test only and with a culture of doubtful purity. *Fusarium acuminatum* E. and E. gave no evidence of parasitism. These results agreed with those of Spaulding (137) in indicating that the ability to attack seedling conifers is not limited to a single species of Fusarium and that *F. moniliforme* is one of the more virulent. The statement by Hartig (61, p. 147–150) that a Fusariumlike fungus was able to corrode the young epidermis of pine seedlings has already been mentioned.

PYTHIUM DEBARYANUM.

Pythium debaryanum Hesse (*Artotrogus debaryanum* Atkinson, *Lucidium pythioides* Lohde) has been known since 1874 (74, 86) as a common cause of damping-off of various angiosperms. The first known observation was made by De Bary about 1864 (74). Despite the large number of hosts on which it has been listed, its parasitism has been definitely established on few. Peters (100) has successfully inoculated sugar beets with pure cultures; at least part of his strains, including presumably part or all of those he used in inoculation tests, were obtained from single spores. Edson (38) working with the same host, reisolated the fungus from inoculated seedlings, and made reinoculations with it. Both find it able to cause root sickness of plants not attacked early enough to be killed outright. Johnson (82) and Knechtel (85) have caused damping-off of tobacco seedlings with it, and the former reported it also able to persist in the cortex and kill the lower leaves of tobacco plants which survived attack. The fungus has long been reputed parasitic on potato tubers and has now been found by Hawkins (70) to be the chief cause of the rot known as "leak" in California. Peters (99) made successful inoculations with pure cultures on cuttings of Pelargonium. Most of the reports of parasitism, however, have been based on microscopic examination or more or less crude inoculation experiments. Noteworthy among the latter are those reported by Hesse (74) on *Camelina sativa* in the original description of the fungus. These were made before pure-culture technique had come into use with fungi, but were so thoroughly checked by microscopic observations at every step in the process that they must be admitted as very good evidence of the parasitism of the fungus. A number of reported angiospermous hosts are listed by Butler (23), Voglino (143), and Johnson (82, p. 34, footnote, and p. 35). Reinking (107) recently reported *Carica papaya* as attacked. A host which the writer has not found in the literature is rice, found by Dr. Haven Metcalf seriously attacked in the seedling stage in a field in South Carolina. A second apparently new host for the fungus is fenugreek (*Trigonella foenum-graecum*). The writer found oospores typical of *Pythium debaryanum* in the tissues of damped-off seed-

lings received by Prof. William T. Horne, from Sonoma County, Calif., with the statement that the disease was seriously affecting the stand. The fungus was easily isolated, and the results of successful inoculations on pine with the cultures obtained are included in Table V (p. 47). A fungus resembling *P. debaryanum* was also found in damped-off cowpea (*Vigna* sp.) seedlings grown in rotation with pines at a Nebraska nursery.

Pythium equiseti Sadebeck, reported as parastic on the prothallia of *Equisetum arvense*, was successfully used by Sadebeck (117) in crude cross-inoculations direct from *E. arvense* to potato tubers. De Bary (5) reversed the direction of the experiment between cryptogamous and phanerogamous hosts by successfully inoculating prothallia of *Equisetum arvense* with *Pythium debaryanum* directly from diseased Lepidium seedlings. He also secured positive results on prothallia of the fern *Todea africana* by the same method. The Equisetum prothallia he found to be especially favorable media on which to develop *Pythium debaryanum*. Fischer considers the fungus found by Bruchmann (17) and Goebel (49) on prothallia of *Lycopodium* sp. as probably identical with *P. debaryanum*. A careful reading of the original articles is sufficient to show that the symbiotic fungus which they described was an entirely different organism. *Saprolegnia schachtii* and *Sporodospora jungermanniae*, reported on two of the Hepaticæ, are of doubtful position (42, p. 403), though Butler (23, p. 89), after a survey of the literature, apparently favors the view that the former is distinct from the damping-off fungus. De Bary (5) reported Vaucheria and Spirogyra apparently immune against *P. debaryanum*.

Early references to *Pythium debaryanum* in connection with gynosperms seem to have been based on the probability that it would be found to be the cause of damping-off in conifers (6; 97; 134, p. 27). The first actual finding of the fungus in any gymnosperms of which the writer is aware is indicated by a label marked *Pythium debaryanum* in the handwriting of Mrs. Flora W. Patterson on a package of coniferous seedlings from a New York nursery collected in 1904.[4] The seedlings, judging from the several rather long cotyledons and the fact that both cotyledons and primary leaves are denticulate, are probably of one of the species of Pinus having medium-sized seed. In 1908 Dr. R. J. Pool, of the University of Nebraska, and his student, Mr. H. S. Stevenson, obtained in culture from damped-off coniferous seedlings a nonseptate fungus which was probably *Pythium debaryanum*, but which formed no distinctive spores on the media on which it was grown. A year later the writer obtained the fungus from pine seedlings at the same nursery and reported it as

[4] In the Office of Pathological Collections, United States Bureau of Plant Industry.

parasitic on pines in preliminary inoculation experiments (62). In 1910 Spaulding (137) found it on spruce in New York, and Hofmann (76) later made successful inoculations on both pine and spruce seedlings, using *P. debaryanum* cultures both from aerial trap plates and from recently damped-off seedlings of cabbage, radish, and Russian thistle (*Salsola tragus*). Hofmann's work, detailed notes of which the writer has been permitted to examine, was done with cultures which were contaminated by molds, but was checked up by microscopic examination of the lesions resulting, which showed the affected tissues filled with nonseptate hyphæ. His results are taken as a rather strong indication that *P. debaryanum* attacks spruce as well as pine and that the fungus attacking conifers is physiologically as well as morphologically identical with that causing the damping-off of angiosperms.

There thus appears from the literature to be reason to believe that *Pythium debaryanum* is parasitic on representatives of two groups of the Pteridophyta and on gymnosperms, as well as on various monocotyledons and dicotyledons, a range of hosts not only remarkable but perhaps unequaled in our present knowledge of plant parasites. Final published proof of parasitism seems to be available for three or four species of dicotyledons only. Additional inoculations on conifers with strains isolated from various other hosts are reported in the present bulletin. Some of the detailed evidence necessary for complete proof of the parasitism of the Pythium on conifers, lacking in experiments previously reported because of the doubtful purity of the cultures used and failure to reisolate and reinoculate with the organism, is also given here, together with evidence of the ability of the parasite to cause root sickness of pines too old to suffer from damping-off.

Descriptive data of interest on *Pythium debaryanum* have been supplied by Hesse (74), De Bary (5), Ward (144), Miyake (89), Butler (23), and Butler and Kulkarni (24). An important contribution to the physiology of the fungus and the factors controlling its passage through the tissues of one of its hosts has recently been made in the previously mentioned paper of Hawkins and Harvey (71).

IDENTITY AND ISOLATION.

The fungus in the writer's cultures referred to *Pythium debaryanum* Hesse has been so called for the following reasons:

(1) The morphological characters agree with those described and figured for *Pythium debaryanum* by other workers and with those of strains obtained from Dr. H. A. Edson under this name.

(2) The absence of zoospores in the writer's cultures agrees with the experience of others with *Pythium debaryanum* (2, 5, 23, 24, 38, 100), all workers with pure cultures having obtained zoospores infrequently, if at all. The earliest work by Hesse (74) in which zoospores were apparently produced

readily at certain times of the year was done before the development of pure-culture methods. Water cultures kept in the dark and in the light, at constant and at varying temperatures, with nutrient substrata consisting of steamed or autoclaved fragments of potato, carrot, sweet potato, turnip, sugar beet, corn meal or rice, nutrient agar, sugar-beet seedlings, and insects have all produced only sexual fruiting bodies and chlamydospores (the so-called conidia).

(3) The successful cross-inoculations, those which Edson (38) used on sugar-beet strains and the writer had found parasitic on pine and had used on pine, strains which Hawkins had found parasitic on potato tubers and Edson on sugar beets, confirm the work of Hofmann (77) in indicating that the Pythium which causes the damping-off pine is a parasite on entirely unrelated species of host plants, a commonly recognized characteristic of *Pythium debaryanum*.

The organism is easily isolated from recently damped-off coniferous seedlings or from soil direct by placing the seedlings or a lump of soil at the edge of a Petri dish of solidified prune agar and transferring to tubes mycelium from the advancing edge of the resulting growth. It has been found in or obtained from damped-off conifers in California, Kansas, Nebraska, Minnesota, and the District of Columbia, as well as in cultures made by Mr. Glenn G. Hahn in Michigan. *Picea engelmanni*, *P. sitchensis*, *Tsuga mertensiana*, *Pinus nigra austriaca*, and *Pseudotsuga taxifolia* are the coniferous hosts from which cultures of *Pythium debaryanum* have been obtained. It has been isolated directly from soil not only in coniferous seed beds but from open grassland in California not adjacent to any seed bed or cultivated crop. Unless Mucor is abundant, Pythium is commonly obtained in apparently pure condition on the first transfer from the plate, as prune agar appears unfavorable for most bacterial growth while allowing rapid spread of the Pythium. On media made from prunes which taste sweet and with a total gross weight of not more than 40 or 50 grams per liter of medium, the Pythium will make a rapid growth, often extending radially 1 mm. per hour at temperatures in the neighborhood of 22° C. and produce both chlamydospores and oospores. A less valuable medium for isolation work, but more convenient for subcultures than any other which has been tested, is autoclaved corn-meal agar. The growth is not luxuriant, but spores are always formed and the cultures seem to be as long lived as those on any other medium, retransfer being rarely necessary more often than twice a year. Much stronger growth and more abundant fruiting is obtained on such media as sugar-beet or rice-stem agar, but the leathery surface of the culture on such media makes transferring difficult. On rice grains, corn-meal mush, beef agar, and on corn-meal agar plus 2 per cent dextose or sucrose no spores are formed and the cultures are short lived, though growth is heavy and on the last-named medium extremely rapid. On agar containing the juice from sour prunes or on corn-meal agar prepared without subjecting it to the high temperature of the autoclave, both growth and fruiting have been very poor or even lacking.

In both artificial cultures and in the tissues of coniferous and dicotyledonous hosts the numerous strains observed showed no conspicuous differences in the size or other characters of the spores produced, though noticeable and constant abnormality was found in one strain in the readiness with which spores were produced and in two strains in the ratio between chlamydospores and oospores in agar cultures. In the first-mentioned strain, obtained from pine in Kansas, and in cultures reisolated from seedlings inoculated with it, both chlamydospores and oospores are produced tardily and so scantily that it is often difficult to find them. In most strains, on the other hand, almost the entire contents of the mycelium are promptly emptied into the spores as soon as the limits of rapid vegetative growth are reached. In another abnormal strain from pine from the same locality, and in still another furnished by Hawkins from a California potato, chlamydospores are produced in large numbers, but oospores are few. In many other strains, including several from California, the opposite condition obtains, oospores in plate cultures being decidedly more numerous than chlamydospores. These peculiarities of particular strains seem to be fairly constant characters, the first abnormal strain mentioned having been under observation for more than three years without any change in its tendency to scanty fruiting, and the low ratio of oospores to chlamydospores having been constant during the shorter periods over which the observation of the other strains extended. In view of the small variation between different strains in the matter of speed of growth, a purely vegetative character, this variation in reproductive habit is somewhat surprising. The strain which produced spores infrequently was unquestionably parasitic, though it killed fewer seedlings than the average *Pythium debaryanum* strains. The strains with the high ratio of chlamydospores were both of at least average virulence on pine.

Oospores in the strains the writer has had in culture, whether examined in agar, in water cultures, or in root tissues, have ordinarily been somewhat larger than the diameter of 14 or 15 to 18 μ given in a number of the descriptions. The maximum range has been 12.8 to 20.6 μ, the same strain sometimes being well down within the usual size range and sometimes ranging from 17 to 20 μ. The largest oogones observed were 26 μ in diameter. Various stages of fertilization are shown in Plate I, figures 2 to 4. Chlamydospores attain a maximum diameter in the case of the limoniform intercalary forms of 32 μ, and spherical chlamydospores sometimes reach a diameter of 28 μ. There is no lower limit for these bodies, as under unfavorable conditions—e. g., in sour-prune agar—they are sometimes all less than 15 μ in diameter, and the smaller ones are little larger than the

hyphæ which bear them. Both oogones and chlamydospores may be either terminal or intercalary.

The normal hyphæ are large, varying from 3 to 7 μ and sometimes more in diameter. Typical hyphæ, showing the false septa developed at the boundary of the protoplasm and the portions of the hyphæ which have been evacuated in the extension of the younger parts, are shown in Plate I, figure 1. At points at which the ends of hyphæ come in contact with the glass of the culture dish, peculiar contact swellings are produced (Pl. I, figs. 5 to 7), much the shape and size of antheridia, but not walled off from the adjacent hyphæ and having no apparent significance in the life history of the fungus. These are not always terminal (Pl. I, fig. 8). It is noteworthy that Hesse described contact swellings at the tips of the hyphæ just before penetrating the epidermis of *Camelina sativa*.

The asexual nonsporangial fruiting bodies of *Pythium debaryanum* are referred to as chlamydospores rather than as conidia, though in most of the previous literature the latter term has been used for them. Hesse called the terminal bodies conidia and the intercalary, gemmæ (74). It is believed that the best terminology and the one which should be followed for all fungi, as it now is for most, is that which limits the term conidium to a spore which is adapted primarily for aerial distribution or which is at least readily separated as soon as it is mature from the parent hypha from which it arises. The most typical conidium, in fact, is a spore which is abstricted by the parent hypha at maturity. The asexual spores of this Pythium remain attached to the parent hyphæ indefinitely even after the hyphæ are dead and empty. It is a common thing to find numbers of these bodies in water cultures, still attached to hyphæ which are so completely empty that it is only with favorable lighting that their thin colorless walls can be seen. So firm is the attachment that vigorous shaking is required to release any considerable proportion of the spores. It seems probable that in nature the spores are released chiefly as a result of the destruction of the hyphæ walls by bacteria. While there is reason to think that *Pythium debaryanum* is sometimes disseminated by wind, it is by no means certain that it is through the medium of these spores. It is true that these bodies have thinner walls than are commonly found in chlamydospores of some other fungi, but they have somewhat thickened walls as compared with the vegetative hyphæ, and they are commonly intercalary. These facts, and the indications that they are better able to withstand unfavorable conditions than are the hyphæ, all tend to entitle them to rank as chlamydospores. De Bary (5) speaks of them as "dauerconidia." Their ability to stand drying is not entirely demonstrated, but is indicated by the relative longevity of the fungus on different media. On beef agar and on rice, on which no spores are

PYTHIUM DEBARYANUM FROM ARTIFICIAL CULTURES.

FIG. 1.—Hyphæ, showing old portions of hyphæ and false septa separating them from the portions still containing protoplasm. FIGS. 2 to 4.—Various stages of oospore formation. FIGS. 5 to 8.—Hyphal swellings at points of contact with glass. From camera-lucida drawings.

formed, a few tests indicate that the fungus is very short lived, sometimes dying in a month. On media on which spores are produced, transfers any time before the sixth month, and often as late as the tenth month, start immediate growth on fresh media. This is true even for strains which produce few or no oospores. The immediate commencement of growth from cultures 3 or 4 months old is taken as an indication that the new growth results from the asexual spores, as oospores are commonly believed to require a resting period of five or more months before they are able to germinate (5, 38).

INOCULATION ON STERILIZED SOIL.

Pythium debaryanum has been used in inoculation in pots of recently autoclaved soil in 16 different series of tests. In 10 of these, fragments of agar cultures were scattered over about one-fourth of the area at the side of each pot when seed was sown; in 2 of these 10 and also in 2 other tests some pots were inoculated over their entire surface. In every one of these 12 heavily inoculated series positive results were indicated by smaller emergence and where any considerable number of sprouting seeds escaped the fungus by heavier damping-off loss in the inoculated pots than in the controls. In some cases the fungus killed all or practically all of the seed or seedlings in the inoculated pots before they emerged from the soil.

In a total of 7 series, part or all of the pots received lighter inoculations, consisting of one or two small fragments of an agar culture placed just below the surface of the soil at the edge of each pot. In 5 of these success was indicated. In the sixth and seventh also of these lightly inoculated sets, there was more damping-off in the inoculated pots than in the controls, but the difference was negligible. The damping-off caused by light inoculations was in general distinctly less than that resulting from broadcast inoculations. To sum up the evidence: Sixteen separate experiments were conducted with *Pythium debaryanum* on pine seedlings in autoclaved soil, and in every one fewer seedlings survived in the inoculated pots than in the checks; the difference in most of the experiments was large.

Of the successful inoculation experiments—that is, those in which the difference between the inoculated pots and the checks seemed significant—9 series included jack pine (*Pinus banksiana*), 7 series western yellow pine (*P. ponderosa*, Colorado and New Mexico seed), and 3 series red pine (*P. resinosa*). In addition to the pines, Douglas fir (*Pseudotsuga taxifolia*, Colorado seed) was grown in two large plats in one of the earlier series, one being inoculated over its entire surface with *Pythium debaryanum*. Because of the poor quality of the seed in the test on Douglas fir, too few seedlings were obtained to furnish a decisive test, but the difference in the emergence in the inoculated plat and the control affords preliminary evidence that

Pythium can cause the "germination-loss" type of damping-off in Douglas fir as well as in species of Pinus. Of the seeds sown in the control plat 13 produced seedlings which appeared above the soil, while only two seedlings appeared from an equal number of seeds sown in the inoculated plat.

Altogether, 38 strains, excluding reisolations, have been tested on one or more of the 3 pine species. Strains from both the Pacific coast and the eastern United States and from a number of hosts other than pines were among those used. With the exception of two or three strains from a pine nursery in Michigan, the use of which was followed by so little damping-off as to leave their parasitism uncertain, all of the strains proved parasitic under favorable conditions, though some were more virulent than others. The positive results in the 14 successful experiments are based on the comparison of a total of approximately 1,160 inoculated pots with 195 control pots.

TABLE III.—*Inoculation experiments with Pythium debaryanum in pots of sterilized soil.*

Series, experiment number, and host.	Pythium strain.		Number of pots.	Inoculation method.	Results.		
	No.	Initial strain from which it was reisolated.			Emerged (per 5-pot unit).	Damping-off after germination (per cent).	Survival (per 5-pot unit).
SERIES A.—Initial inoculations:							
No. 58, Pinus banksiana	218		5	Agar cultures broadcast at one side of pot.	1	100	0
	Controls.		5	No inoculum	74	0	74
	do		5	do	82	0	82
No. 58, Pinus ponderosa	295		5	Agar cultures at single point in each pot.	41	28	30
	Controls.		5	No inoculum	55	0	55
No. 62, Pinus banksiana	258		5	Agar cultures broadcast at one side of pot.	16	81	3
	do		5	do	18	89	2
	Controls.		5	No inoculum.	90	0	90
	do		5	do	82	0	82
	do		6	do	82	0	82
	338[a]		5	Agar cultures broadcast at one side of pot.	14	72	4
	347[a]		5	do	3	67	1
	348[a]		5	do	13	67	4
	Control[a]		5	No inoculum	78	0	78
SERIES B.—Reisolated strains:							
No. 62, Pinus banksiana	338	No. 295 (in P. ponderosa, expt. 58).	5	(b)	14	72	4
	Controls.		5		78	0	78

[a] A different soil used in these pots from that used with strain 258 and the first three control units.

[b] All inoculations with fragments of agar cultures scattered broadcast at one side of the pot, including about one-fourth of its area. Nothing was added to the controls in experiment 62, but sterile agar was added to the controls in experiments 66, 67, and 68.

TABLE III.—*Inoculation experiments with Pythium debaryanum in pots of sterilized soil*—Continued.

Series, experiment number, and host.	Pythium strain.		Number of pots.	Inoculation method.	Results.		
	No.	Initial strain from which it was reisolated.			Emerged (per 5-pot unit).	Damping-off after germination (per cent).	Survival (per 5-pot unit).
Series B.—Reisolated strains—Continued.							
No. 66, Pinus banksiana	338	No. 295 (in P. ponderosa, expt. 58).	5	(a)	9	67	3
	345	No. 218 (in P. banksiana, expt. 58).	5	(a)	15	33	10
	414	No. 258 (in P. banksiana, expt. 62, 2d unit).	5	(a)	36	25	27
	415	do	5	(a)	59	10	54
	419	No. 348 (in P. banksiana, expt. 62).	5	(a)	25	100	0
	450	No. 347 (in P. banksiana, expt. 62).	5	(a)	41	72	11
	Controls		25		75	14	64
No. 67, Pinus banksiana	338	No. 295 (in P. ponderosa, expt. 58).	5	(a)	7	57	3
	345	No. 218 (in P. banksiana, expt. 58).	5	(a)	24	37	15
	414	No. 258 (in P. banksiana, expt. 62, 2d unit).	5	(a)	57	24	44
	415	No. 258 (in P. banksiana, expt. 62, 1st unit).	5	(a)	30	37	19
	419	No. 348 (in P. banksiana, expt. 62).	5	(a)	62	65	22
	450	No. 347 (in P. banksiana, expt. 62).	5	(a)	53	27	39
	Controls		23		87	5	83
No. 68, Pinus resinosa	338	No. 295 (in P. ponderosa, expt. 58).	5	(a)	85	26	63
	345	No. 218 (in P. banksiana, expt. 58).	5	(a)	76	24	58
	414	No. 258 (in P. banksiana, expt. 62, 2d unit).	5	(a)	98	14	84
	415	No. 258 (in P. banksiana, expt. 62, 1st unit).	5	(a)	92	11	82
	419	No. 348 (in P. banksiana, expt. 62).	5	(a)	95	45	52
	450	No. 347 (in P. banksiana, expt. 62).	5	(a)	84	40	51
	Controls		18		104	0	104

[a] All inoculations with fragments of agar cultures scattered broadcast at one side of the pot, including about one-fourth of its area. Nothing was added to the controls in experiment 62, but sterile agar was added to the controls in experiments 66, 67, and 68.

As has been stated, securing positive results did not always mean that the control pots remain uninfected. Even with the most careful treatment and the use of boiled water throughout the experiment

it proved difficult to keep the control pots entirely free from damping-off. Cultures from seedlings which damped-off spontaneously in control pots indicated that Pythium as well as Fusarium may be introduced by accident, even when insects, birds, and rodents are excluded. This agrees with the evidence of Hofmann (77) that *Pythium debaryanum* is sometimes disseminated by wind, despite its apparent lack of adaptation to wind distribution. It is also indicated, however, that unheated tap water increases damping-off when used on control pots and probably carries this semiaquatic fungus. Notwithstanding infections in the controls of a number of the experiments, it is believed that the large number of pots whose results have been considered in drawing conclusions, the fact that the Pythium pots lost more heavily than the controls in every one of the 16 experiments, and the magnitude of the differences between both the emergence and subsequent damping-off figures for the inoculated pots and the controls in most of the experiments establish the parasitism of the fungus in inoculation on autoclaved soil without it being necessary to present all the evidence in detail. The pot series which involved reisolation and reinoculation (Table III), together with the results given for other purposes in Tables V and VI, seem sufficient by themselves to establish a parasitic relationship.

REISOLATION AND REINOCULATION.

In a number of the experiments dead seedlings in the inoculated pots were examined and typical Pythium hyphæ and spores were found. In three of the experiments in which the controls remained entirely free from disease up to the time the experiment was closed, reisolations and reinoculations were made in accordance with the usual rules of proof. The results are given in Table III.

From Table III it will be seen that five strains reisolated from *Pinus banksiana* and one strain reisolated from *P. ponderosa* gave positive results in pots of *P. banksiana* and *P. resinosa*. In addition to the reinoculations shown in the table, the strain reisolated from *Pinus ponderosa* (No. 338) was again reisolated in duplicate from *P. banksiana* in experiment 62, and both these secondary reisolations gave cultures which were parasitic on *P. banksiana* and *P. resinosa* in subsequent inoculations.

That the organisms reisolated were actually the same as those used in the initial inoculation is indicated not only by the absence of disease in the control pots of experiments 58 and 62, but by the distinctive characters of some of the strains. In general, cultures reisolated from strongly parasitic initial strains were themselves strongly parasitic and vice versa. This is shown by comparing the figures for the initial and reisolated strains, as shown in Table IV. Each figure represents the average results in 10 pots of jack pine and 5 of red

pine in experiments 66, 67, and 68 combined. The figures are relative, the mean survival of 47 different strains used in all three experiments being taken as 10. A survival figure above 10 therefore means that the strain was less destructive than the average Pythium, and a figure below 10 indicates more than average virulence. As strain 218 was not used in these three experiments, strain 345 can not be compared.

TABLE IV.—*Comparative virulence of original cultures and reisolated strains of Pythium debaryanum in experiments 66, 67, and 68.*

Pythium strain.	Description.	Relative survival.	Pythium strain.	Description.	Relative survival.
No. 258	Original culture	16	No 409	Reisolated from 338	9
No. 414	Reisolated from 258	12	No. 347	Original culture	4
No. 415	do	12	No. 450	Reisolated from 347	7
No. 295	Original culture	4	No. 348	Original culture	10
No. 338	Reisolated from 295	4	No. 419	Reisolated from 348	4
No. 408	Reisolated from 338	6			

These figures are not absolutely consistent, but are to be viewed as contributing to the evidence furnished by the absence of damping-off in the control of experiments 58 and 62 that the cultures reisolated in those experiments were actually identical with the original strains. A further proof of this identity is in the fruiting tendencies of the strains. Both Nos. 414 and 415, the strains reisolated from original strain 258, exhibited the peculiarly sparse spore production which has been characteristic of strain 258 for the entire period during which it has been in culture. The other reisolated strains, taken from pots inoculated with normally fruiting strains, all showed normal spore production.

PURITY OF CULTURES.

A slight deficiency in the evidence as to the parasitism of *Pythium debaryanum* both in the writer's work and apparently in all previous investigations except those of Peters (100) and possibly Knechtel [5] is the lack of single-spore cultures. The large number of strains which have remained apparently pure through numerous subcultures and have retained their individual characteristics as to virulence and fruiting tendencies (one strain having been carried on artificial media continuously for eight years without material change) give very strong justification for believing that the cultures used were pure. In three early inoculation tests the cultures used were afterwards found to have been contaminated by bacteria carried by mites; the positive results obtained in these three were the basis of the ear-

[5] Knechtel's work in Rumanian has been available to the writer only in the German abstract, which makes an ambiguous statement on this point.

liest report of pathogenicity (62), but have not been used as evidence in the present bulletin, though the contaminating bacteria in one of them, when tested independently, showed no evidence of parasitism. In all the experiments mentioned in the foregoing as giving positive results with Pythium the cultures used were apparently pure.

Cultures from single chlamydospores should be reasonably easy to secure, part of the chlamydospores in water cultures being separable from the mycelium by vigorous shaking, and further inoculation tests with cultures so obtained are probably desirable. The experiments so far conducted are believed to be sufficiently conclusive, however, for all practical purposes. For isolation of absolutely pure lines of this or any other cœnocytic fungus, it is evident, as pointed out by Dr. W. H. Weston (146), that isolations should be made from the uninucleate swarm spores. For the determination of the bare fact of pathogenicity such a refinement would be superfluous.

CROSS-INOCULATIONS.

The physiological identity of the Pythium attacking coniferous seedlings with the one which attacks dicotyledons is indicated by the results of several inoculation experiments. The last two experiments, one with jack pine and one with red pine for the host, are the most comprehensive and give results sufficiently decisive so that quotation of the corroborative evidence from earlier experiments is considered unnecessary. The results appear in Table V. Each unit consisted of five 3-inch pots except in the controls, in which 23 pots were used in the jack-pine experiment and 18 in that with red pine. In the second experiment, separate records were kept of the survival in each pot, and the probable error calculated from the controls was less than two seedlings per pot for a single pot, less than 0.9 for a mean of 5 pots, and less than 0.5 for the mean of the 18 control pots. While the number of controls was, of course, insufficient to furnish an exact basis for such a calculation, the small value found tends to confirm the impression gained from inspection of the table that considerable confidence can be placed in the results.

The difference appearing in Table V between jack pine and red pine in point of susceptibility to germination loss from Pythium agrees with field observations in Nebraska, the red pine at the Bessey Nursery, though on the whole more susceptible than jack pine to damping-off losses, having given indication of more resistance to the disease for the first week or two. Inoculations in other experiments on western yellow pine indicate that the strains which attack it are identical with those attacking jack pine and red pine.

The conclusion reached from the cross-inoculation results is that the Pythium causing damping-off of the three species of pine men-

tioned is identical with *Pythium debaryanum*, causing leak of potato tubers and the damping-off of seedlings of two dicotyledonous families.

TABLE V.—*Results of inoculations on jack pine and red pine with Pythium debaryanum from various hosts.*

Strain.	Host from which isolated.	Inoculation results.					
		On jack pine.			On red pine.		
		Emerged (per 5-pot unit).	Damping-off (per cent).	Survival (per 5-pot unit).	Emerged (per 5-pot unit).	Damping-off (per cent).	Survival (per pot).
	Dicotyledons:						
No. 131 [a]	Potato tuber	45	36	30	101	23	15.6
No. 810 [b]	Do	45	34	30	62	41	7.4
	Average potato	45	35	30	82	32	11.6
No. 294 [c]	Sugar-beet seedlings	50	8	46	79	36	10.2
No. 295 [c]	Originally potato strain 131, but twice inoculated on and reisolated from sugar-beet seedlings by Edson	28	32	19	62	48	6.6
No. 296 [d]	Sugar-beet seedlings	19	32	13	68	58	5.8
	Average, sugar beet	32	24	26	70	47	7.4
No. 529	Fenugreek seedlings [d]	36	31	25	102	26	15.0
No. 530	Do	60	49	31	108	22	16.8
	Average, fenugreek	48	40	28	105	24	16.0
	Conifers:						
No. 258	Western yellow-pine seedlings	58	9	53	109	17	18.0
No. 550	Sitka spruce seedlings	15	80	3	39	98	.2
No. 555	Engelmann spruce seedlings	42	29	30	45	70	5.0
	Average, spruces	29	55	17	42	84	2.6
	Controls	87	5	83	104	0	20.9

[a] Furnished by Mrs. C. R. Tillotson; has been used successfully on sugar-beet seedlings by Dr. H. A. Edson.
[b] Furnished by Dr. L. A. Hawkins: cause of leak.
[c] Furnished by Dr. H. A. Edson.
[d] Diseased material furnished by Prof. W. T. Horne.

VARIATIONS IN VIRULENCE OF PYTHIUM STRAINS ON PINE.

In *Pythium debaryanum* strains, as in the case of *Corticium vagum*, there appeared to be a considerable difference in the parasitic activity of different strains used in the same experiment. Figures 14, 15, and 16 show graphically the results from inoculations with different strains of *P. debaryanum* in all the experiments in which it was possible to compare directly the activity of different strains. All the inoculations involved at the time of sowing the addition to the soil of cultures on nutrient media in recently autoclaved 3-inch pots. In experiment 31C the inoculum fragments were scattered over the whole pot, in 31D at only one point in each pot, and in the others were distributed over about one-fourth of the pot's area. As noted elsewhere, the variations observed in the results may have been due in part to differences in the ability of the different strains to main-

tain themselves saprophytically in the soil used rather than entirely due to difference in virulence.

The data shown in figures 15 and 16 indicate in the first place rather more accidental variations in the results with Pythium than with Corticium (see figs. 1, 2, 10, and 11). The agreement between original and reisolated strains from the same original source is decidedly less good than in the case of Corticium (see experiments 71 and 72, figs. 10 and 11). In general, there are only two strains of

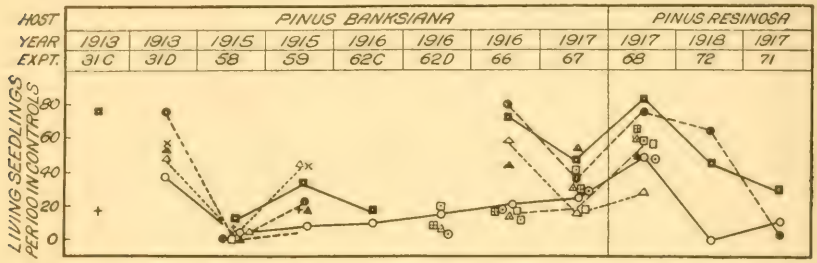

FIG. 14.—Diagram showing variations in virulence as indicated by the living seedlings in pots of autoclaved soil inoculated with different strains of *Pythium debaryanum*. For experiments Nos. 31 and 66 to 72, inclusive, the surviving seedlings at the end of two or three weeks after germination are shown. For the other experiments damping-off was so heavy in the inoculated pots that the survivals did not give differential results for the different strains, and the germinations are therefore shown. The reports are based for experiments Nos. 71 and 72 on 2 or 3 pots for each strain in each experiment, and for the other experiments on not less than 5 pots for each strain. In experiments Nos. 66, 67, and 68 the number of pots in each experiment for the strains whose reisolations were also used varied from 10 to 40 for each strain, the results of the separate 5-pot units being shown in figure 15. The strains indicated by the different symbols are as follows: *From potato:* ●=Strain 131, isolated in 1909, California. Furnished by Mrs. C. R. Tillotson. *From sugar beet:* ○=Strain 295 and its reisolations from pine. No. 295 was furnished by Dr. H. A. Edson as a reisolation of strain 131, after having been passed by him through two generations of sugar-beet seedlings. ▲=Strain 294, isolated in 1912. Furnished by Dr. Edson. △=Strain 296, isolated in 1912, Wisconsin. Furnished by Dr. Edson. ×=Strain 297, originally from pine, Nebraska, 1911. Passed through two generations of sugar-beet seedlings by Dr. Edson. *From pine seedlings:* +=Strain 255, Kansas, 1913. Chlamydospores numerous; oospores rare. ■=Strain 258 and its reisolations, Kansas, 1913. A sparsely fruiting strain. □=Strain 218 and its reisolation, Kansas, 1912. ⊙=Strain 347 and its reisolation, Washington, D. C., 1915. ⊡=Strain 348 and its reisolation, Washington, D. C., 1915. ◬=Strain 349, Washington, D. C., 1915. ⊞=Strain 354, Minnesota, 1915.

Pythium which can be said to have definitely shown difference in activity continuing through several years and on different species of pine. These are strains 295 and 258. As No. 258, the weak strain, has also been found abnormal in its fruiting tendencies, the evidence in these graphs does not indicate a decided difference in virulence between different typical strains of *Pythium debaryanum*. The other strain, which seems rather uniformly weaker than No. 295, is No. 131, which according to Dr. Edson's records was originally the

same strain, No. 131 having been twice used in his inoculation experiments on sugar beets and strain 295 recovered from the second experiment. The apparent difference between this original strain and its supposed reisolation may possibly be due to the treatment given strain 131. Before it was used in any of the experiments shown but after it had been used by Dr. Edson, it was allowed to get very dry and was revived with great difficulty, growth being very slow. While it apparently recovered all of its normal growth qualities after one or two transfers, it is thought that this may possibly explain the apparently decreased virulence in the later experiments.

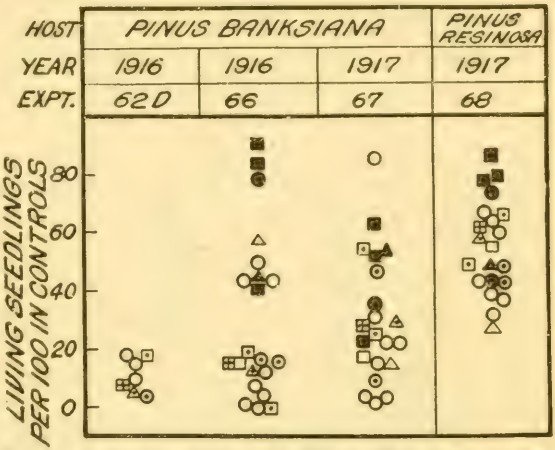

FIG. 15.—Diagram showing the results of inoculations with strains of *Pythium debaryanum*. This figure supplements figure 14, giving the results for original and reisolated strains independently. Each point plotted is based on the results in five pots. The object of this diagram is to give an idea of the degree of variability in the success of inoculations. An explanation of the symbols used will be found in the legend of figure 14.

The failure to secure as definite indications of constant virulence differences as were obtained for several of the Corticium strains is believed to be in part due to a smaller actual difference between the different Pythium strains appearing in the graphs and in part to a larger accidental variation between results in pots inoculated with the same strain. The growth of Pythium on agar media is much more affected by variations in the substratum than is the growth of Corticium, and it is rather natural to expect greater variations when the two fungi are added to autoclaved soil. In experiments 66, 67, and 68 a number of strains not used in the earlier experiments were tested, in addition to the strains previously used. The survival results for all the different strains, both original and reisolated, 47 in all, are shown graphically in figure 16. The results in experiments 66 and 67, both on *Pinus banksiana*, are averaged and taken as the subject, while the results with the same strains in experiment 68 are made relative and shown by the broken line. The correlation between the performance of the same strains on the two species of pine is by no means as clear in the graph as it was in the case of the Corticium strains (fig. 11). The areas bounded by the broken line and

the horizontal line showing the location of the mean for experiment 68 are much larger below the means than above it in the left-hand portion of the graph, while the reverse is true in the right-hand portion. To this extent the relative activity of the strains in this experiment agrees with the performance of the same strains in the two jack-pine experiments, as shown by the solid line. It can not be decided from an inspection of the graph whether there is a real agreement, in view of the large accidental variation present. However, the correlation coefficient, 0.446 ± 0.079, five and one-half times its probable error, indicates a considerable correlation, not as good as was found for the Corticium strains, but sufficient to establish a strong presumption that observed differences in activity of the dif-

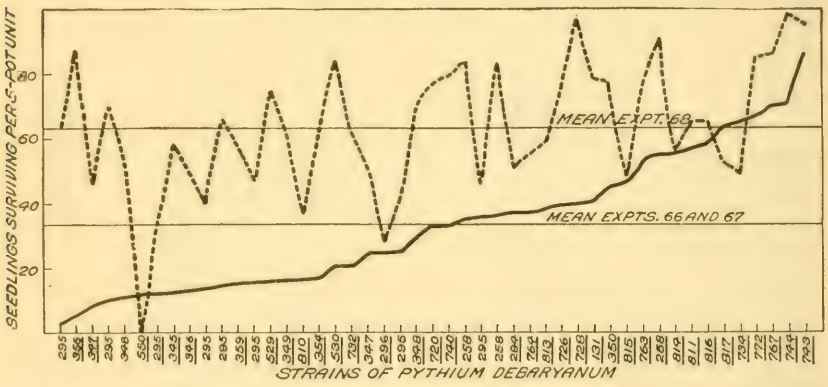

FIG. 16.—Diagram showing the comparative virulence of 47 strains of *Pythium debaryanum* in successive inoculation experiments on species of Pinus. The results in experiments Nos. 66 and 67 (on *Pinus banksiana*) are shown by the solid line, the strains being arranged from left to right in the order of descending virulence indicated by the number of seedlings surviving in those experiments. The results from the use of the same strains in experiment No. 68 (on *Pinus resinosa*) are shown by the broken line. Such correlation as there is between the two curves (coefficient 0.45 ± 0.08) goes to indicate a real difference in virulence between the different strains. The strains indicated by the underscored numbers are original strains, and those not underscored are reisolations from the original strains in earlier inoculation experiments on pine seedlings.

ferent strains in these inoculation experiments were in part actually due to differences in the capacity of the strains.

It has been suggested in the foregoing that the difficulty in demonstrating constancy in the difference in virulence between the various strains of *Pythium debaryanum* is due in part to the lack of such extreme differences as were observed between the various Corticium strains. Figure 13 shows the distribution of the different original Pythium strains according to the virulence indicated in the three inoculation experiments of figure 16 (application to autoclaved soil at the time of sowing). Each value plotted is based on the average results in 15 pots. Of the strains used, 21 were from species of pine, 1 from spruce, 2 from potato tubers, 2 from fenugreek, 3 from sugar beet, and 6 from soil direct. Despite the considerable number of

strains, they are not much more representative than the smaller number of Corticium strains experimented with. All of the strains from soil direct and 11 of the strains from pine were taken at approximately the same time from the same nursery in Michigan by Mr. Glenn G. Hahn; despite the fact that these were the most recently isolated of the strains used, nearly all of them proved weak in the inoculations. Of the 17 strains which proved the weakest (out of 35), all but 3 were from this Michigan nursery. The 18 strains from other sources (5 from California, 2 from Minnesota, 2 from Kansas, 1 from Wisconsin, 2 from an unknown locality, and 6 from Washington, D. C., representing two coniferous and three dicotyledonous host genera), as shown by solid circles in figure 13, for the most part were rather closely grouped within the more virulent portion of the range. The coefficient of variability in the survivals allowed by the 35 *Pythium debaryanum* strains is 39±3.6 per cent, while for the smaller number of original strains of *Corticium vagum* in experiments 71 and 72 it is 63±9.7 per cent. It is evident from figure 13 that if there had not been a disproportionately larger number of strains from the Michigan nursery the variability of the *P. debaryanum* strains would have been much less than 39 per cent. The number of strains was, of course, altogether insufficient for either fungus to represent adequately a population as immense as the total number of strains of either of these omnipresent species. The above data, however, contain the only available information of which the writer is aware on variation in the virulence of different strains of *P. debaryanum*.

The evidence as a whole, both from the results shown in figure 13 and the experience with 6 other strains which were not used in the experiments on which figure 13 was based, lead the writer to believe that most strains of *Pythium debaryanum* taken from lesions in plants are ordinarily likely to prove rather virulent parasites on pine seedlings. It further appears that the variation in virulence between the different strains of *P. debaryanum* on pine seedlings is less than the variation in strains of *Corticium vagum*.

PYTHIUM INOCULATIONS ON UNHEATED SOIL.

Inoculations with *Pythium debaryanum* were made in western Kansas on a fine sand containing little humus after treating the soil with acid followed by lime. Commercial sulphuric acid was applied at the rate of 14.8 c. c. per square foot of bed, followed two days later by 25.5 grams of air-slaked lime raked into the soil (0.16 liter of acid and 0.274 kg. of lime per square meter). The acid was diluted before applying with 256 volumes of water. The seeds were sown in drills, and inoculum was placed in the drills at the time of sowing. Each unit involved approximately 11 linear inches of drill, and all

received equal quantities of seed. Three strongly parasitic strains of Pythium were used, and a total of 12 units of jack pine and an equal number of western yellow pine was inoculated with 12 interspersed units of each species as controls. The mean results are as follows:

Pinus banksiana.—Inoculated plats: Emerged, 64.2±4.9; died during the next 17 days, 25 per cent. Control plats: Emerged, 85.5±3.6; died during the next 17 days, 13 per cent.

Pinus ponderosa.—Inoculated plats: Emerged, 34.6±1.8; died during the next 9 days, 39 per cent. Control plats: Emerged, 45.4±1.3; died during the next 9 days, 25 per cent.

The difference in emergence apparently due to the inoculation is for the first species three and one-half and for the second nearly five times its probable error. While, of course, 12-unit means are insufficient to allow the calculation of entirely reliable probable errors, they give some idea of the amount of variability of the results and the confidence which can be given them. It is impossible to give any such expression applying directly to the damping-off percentages and their differences, for the reason that averages for this item have been made in the writer's work not by averaging the percentages for the individual units but by totaling all the seedlings and the dead seedlings on the plats to be averaged and recalculating the percentage from these figures. This seems the only safe method, as otherwise units in which germination is low by accident or by the action of parasites will be given an influence on the resultant mean entirely disproportionate to the number of seedlings which they contain. Average values for damping-off obtained by this method and by the method of averaging the percentages of the individual plats or pots are often very different; it not uncommonly happens that the units in which germination is lower than the average also have especially high damping-off percentages, both phenomena being caused by an unusual activity of parasites. In such case to average the percentages themselves usually gives a higher damping-off figure than to total the seedlings for the different units and redetermine the percentage, and the latter practice is considered the better. In the present case the differences in the damping-off percentages obtained by the two methods are not great. The figures obtained by averaging the percentages of the ultimate units are as follows:

Pinus banksiana.—Inoculated, loss 30.9±5.0 per cent; controls, loss 13.2±2.8 per cent.

Pinus ponderosa.—Inoculated, loss 40.0±5.1 per cent; controls, loss 24.1±3.3 per cent.

The differences between the inoculated and control plats in damping-off percentage were for the first species a little over and for the second a little under three times their indicated probable errors.

The results in general make it appear that the Pythium was able to kill some pines both before and after their appearance above the soil surface on the soil treated with the acid and lime. The control in this experiment did not receive the nutrient substratum added with the Pythium inoculum, but an experiment run under the same conditions at nearly the same time, in which seven strains of hyphomycetes with the same substrata entirely failed to decrease survival, indicates that the rice subtratum was not in itself the cause of the observed result. The rather weak action of the Pythium in these experiments stands out in sharp contrast to the results with *Corticium vagum* in the same experiments, practically all emergence being prevented by most of the Corticium strains used, some of which had proved less active than Pythium in tests on autoclaved soil.

In a soil in Nebraska, somewhat similar but with more humus, 5.5 c. c. (three-sixteenths fluid ounce) of sulphuric acid per square foot applied in solution at the time of sowing had been found greatly to decrease damping-off. In different parts of beds treated with acid from 10 to 17 days earlier, 96 plats, each 3 inches square, were laid out, and each plat was inoculated at the center. Interspersed with these were 96 plats set apart as controls. Emergence had already begun at the time of inoculation. Jack pine, red pine, and Corsican pine were the hosts, and three Pythium strains of known parasitism, growing in pieces of prune agar the size of peas, constituted the inoculum. The damping-off after emergence was less than 1 per cent higher for the inoculated plats than for the controls. Even such a light inoculation would probably have given some results in autoclaved soil, so the experiment indicates, as would be expected, that this acid-treated soil was less favorable for *Pythium debaryanum* than steamed soil.

On pots containing entirely untreated soil the following series of inoculations were made at the time of sowing the seed:

Inoculation at one point in each pot:

Experiment 25. Jack and western yellow pine, 1 pot of each inoculated; survival 13 days after emergence slightly greater in both than in the six controls.

Experiment 27. Jack pine, 73 pots, 27 controls; average emergence, 59 in inoculated pots and 56 in controls; damping-off, 39 per cent in inoculated pots and 37 per cent in controls.

Experiment 29. Jack, Corsican, and western yellow pine, 112 plats inoculated just as emergence commenced instead of at seed sowing, as in other cases, and 112 controls alternating with them; damping-off was less in the inoculated plats than in the controls.

Experiment 31. Jack pine, 8 pots inoculated, 8 controls; inoculated, emergence 33 per cent, damping-off 13 per cent, survival 198; controls, emergence 38 per cent, damping-off 26 per cent, survival 196.

Experiment 58A. Jack pine, 5 pots inoculated, 5 controls; inoculated, emergence 59 per cent, damping-off 32 per cent, survival 40; controls, emergence 51 per cent, damping-off 12 per cent, survival 45.

Inoculations at two points in each pot:

Experiment 26A. Jack pine, 3 pots inoculated, 4 controls; inoculated, emergence 29 per cent, as compared with 39 per cent in the controls; subsequent damping-off the same in both.

Inoculations at four points in each pot:

Experiment 58B. Jack pine, 5 pots inoculated, 5 controls; inoculated, emergence 51 per cent, damping-off 10 per cent, survival 46; controls, emergence 43 per cent, damping-off 22 per cent, survival 34.

Experiment 59A. Jack pine, 5 pots inoculated, 5 controls; inoculated, emergence 55 per cent, damping-off 2 per cent, survival 54; controls, emergence 50 per cent, damping-off 8 per cent, survival 46.

Of these experiments, No. 29 was in the original fine sandy soil of a nursery in Nebraska in which Pythium is commonly found native and damping-off losses are usually heavy. Experiments 58A and 59 were conducted on soil from the same source which had been kept dry in the laboratory for five years; experiments 25, 27, 31, and 58A were on greenhouse mixtures of sand and soil. In experiments 31, 58A, 58B, and 59 parallel inoculations were made on autoclaved portions of the same soil, with definitely positive results in three of the four cases. In the heated soil the results were positive, not only because of smaller losses in the controls but because the losses in the inoculated pots were actually heavier in the sterilized soil than in that untreated.

Inoculations broadcast:

Experiment 31. Jack pine, 8 pots inoculated, 8 controls; inoculated, emergence 31 per cent, damping-off 39 per cent, survival 129; controls, emergence 38 per cent, damping-off 26 per cent, survival 196.

Experiment 59. Jack pine, 5 pots inoculated, 5 controls; inoculated, emergence 58 per cent, damping-off 22 per cent, survival 45; controls, emergence 44 per cent, damping-off 2 per cent, survival 43.

Even with these broadcast inoculations the results on untreated soil were too indefinite to allow the drawing of positive conclusions. In both experiments much heavier losses than these resulted from inoculations on steamed soil. It is evident that experiments on sterilized soil do not always show what can be expected on ordinary soil. The same thing is indicated by the results of Edgerton with tomato wilt (36).

CONCLUSIONS AS TO THE PARASITISM OF PYTHIUM DEBARYANUM.

Pythium debaryanum has been found in low-altitude nurseries in all the species of conifers from which a serious effort has been made to obtain it, and its parasitism has been indicated in autoclaved soil on all of the conifers on which inoculation has been attempted. Therefore, although the work reported has been limited to a relatively small number of hosts, it seems likely that it will be found able to cause damping-off in most of the species of the Abietoideæ which suffer seriously from the disease. Just how active as a parasite it is

under ordinary nursery conditions is yet to be proved. The results in inoculations on disinfected soil, together with the frequency with which the fungus has been isolated from seedlings in the nurseries, lead the writer to believe that it is an important cause of disease in the seed beds. Further experiments on unheated soil, however, are considered desirable.

RHEOSPORANGIUM APHANIDERMATUS.

CULTURAL STRAINS.

A culture of a parasite on radishes and sugar beets, described by Edson (39) under the above name, was obtained from him, and another strain, shown by Edson's records to be a subculture from the same original strain, was furnished by the department of plant pathology of the University of Wisconsin. In parallel cultures on solid media this fungus proved in many ways remarkably like *Pythium debaryanum*, reacting in practically the same way to the different media on which it was grown both in relative growth rate and in spore production. Mycelium, chlamydospores, oogones, antheridia, and oospores are not recognizably different from those of *Pythium debaryanum*. The oospores have seemed on the whole slightly larger and the mycelium a little more inclined to aerial growth than most of the *Pythium debaryanum* strains, but neither difference was sufficient to have diagnostic value. Swellings of the hyphæ occurred at points in contact with glass, just as with *Pythium debaryanum* (Pl. I, figs. 5 to 7).

In liquid cultures the Rheosporangium was readily distinguished from Pythium by the formation of the presporangia described by Edson. Autoclaved cylinders of turnip, 15 to 20 mm. long, cut with a 5-mm. cork borer, proved convenient bases for growth of both Rheosporangium and Pythium in water culture and quite as satisfactory as sterilized beet seedlings. Presporangia were also produced in autoclaved soil, and in a single lot of corn-meal agar they were formed abundantly in the agar in Petri dish cultures. In none of the writer's cultures, either with flies, sugar-beet seedlings, or turnip cylinders as nutrient bases, were mature escaped sporangia or swarm spores commonly produced.

The Rheosporangium was not obtained in any of the numerous cultures made from coniferous seedlings or from seed-bed soil.

INOCULATION EXPERIMENTS.

The Rheosporangium cultures above referred to, strain 229 furnished by Dr. Edson and strain 351 received from the University of Wisconsin, were tested on pine and red-beet seedlings, with parallel inoculations with *Pythium debaryanum*. The results appear in Table VI.

TABLE VI.—*Results of parallel inoculation with Rheosporangium aphanidermatus and Pythium debaryanum on pine seedlings in autoclaved soil.*

Experiment number, host, and inoculating fungus.[a]	Number of pots.	Results. Emerged (per cent of seed).	Damping-off (per cent).	Survival (per cent of seed).
No. 30, Pinus ponderosa:		*Per pot.*	*Per pot.*	*Per pot.*
Rheosporangium, strain 229	5	23	3	22
Pythium, 2 strains	5	14	38	9
Controls	5	20	22	16
No. 31, Pinus banksiana:				
Rheosporangium, strain 229	5	45	13	40
Pythium, strain 225	10	12	44	7
Controls	25	43	5	41
		Per 5-pot unit.		*Per 5-pot unit.*
No. 58, Pinus banksiana:				
Rheosporangium, strain 229	5	46	2	45
Pythium, 8 strains	40	2.6	50	1.3
Controls	10	78	0	78
No. 59, Pinus banksiana:				
Rheosporangium, strain 229	5	46	11	41
Pythium, 8 strains	40	15	35	10
Controls	10	64	0	64
No. 61, Pinus banksiana:				
Rheosporangium, strain 351	20	16	36	10
Pythium, 2 strains	20	2	57	1
Controls	20	60	2	59
No. 61, Pinus banksiana and beets in same pots:				
Pines, Rheosporangium, strain 351	5	1	(b)	(b)
Beets, Rheosporangium, strain 351	5	70	88	8
No. 61, beets alone:				
Rheosporangium, strain 351	5	43	72	12
No. 62A, beets:				
Rheosporangium, strain 229	15	89	1	88
Rheosporangium, strain 351	4	42	35	27
Pythium, 2 strains	20	12	83	2
Controls	11	86	0	86
No. 62A, beets:				
Rheosporangium, strain 229	5	50	20	40
Rheosporangium, strain 351	5	c 27	78	c 6
Controls	5	c 77	1	c 76
No. 62A, beets and Pinus banksiana in same pots:				
Pines, Rheosporangium, strain 229	5	64	14	55
Beets, Rheosporangium, strain 229	5	42	22	33
Pines, Rheosporangium, strain 351	5	46	33	31
Beets, Rheosporangium, strain 351	5	27	78	6
Pines, Controls	5	81	0	81
Beets, Controls	5	67	0	57
No. 62B, Jack pine: [d]				
Rheosporangium, strain 229	2	58	14	50
Rheosporangium, strain 351	2	53	10	48
Pythium, strain 258	2	10	100	0
Controls	3	82	0	82
No. 66, Jack pine:				
Rheosporangium, strain 229	5	63	9	58
Rheosporangium, strain 351	5	80	52	39
Pythium, 47 strains and substrains	235	43	33	29
Controls	25	75	14	64
No. 67, Jack pine:				
Rheosporangium, strain 229	5	107	0	107
Rheosporangium, strain 351	5	88	6	83
Pythium, 47 strains and substrains	235	51	26	38
Controls	23	87	5	83
No. 68, Red pine:				
Rheosporangium, strain 229	5	105	0	105
Rheosporangium, strain 351	5	124	0	124
Pythium, 47 strains and substrains	235	86	27	63
Controls	18	104	0	104

[a] Location of the inoculum: In experiment 30, at one point at the edge of each pot; in experiment 31, over the entire pot; in all other experiments, over one-quarter the area of each pot.

[b] The breakage of the one seedling not killed while sprouting prevented the determination of results.

[c] Double seed density in these pots; emergence and survival figures halved to allow direct comparison with other units. This high seed density may explain in part the higher loss in strain 351 than in strain 229.

[d] Experiment 62B was conducted at the same time as 62A, but with a different soil.

Table VI shows that in experiments 30 and 67 the loss was less in the Rheosporangium pots than in the controls and that in experiment 68 the results were entirely negative, while in the remaining seven

experiments the losses were heavier in the Rheosporangium pots. Especially in experiments 61 and 62A the evidence indicates very strongly that both germination loss and subsequent damping-off of the seedlings which come up can be caused by inoculation with Rheosporangium on jack pine under favorable inoculation conditions. It is, however, obvious that in all of the experiments the parallel inoculations with *Pythium debaryanum* gave much more positive results. The Pythium was active under conditions in which the Rheosporangium gave no evidence whatever of parasitic capacity. It furthermore appears that the two strains of Rheosporangium, though probably identical originally, differed in virulence at the time of their comparison in these experiments. The greater virulence of strain 351 was quite distinct in most of the comparative tests on beets as well as on pines. The possibility that the original culture was really a composite of two or more strains, of which different ones survived in the subcultures kept at Washington and Madison, respectively, seems worth considering. Such an accident might also have been responsible for the divergence of Pythium strains 131 and 295 referred to in another section.

Further evidence of the parasitism of Rheosporangium was obtained in inoculations with cultures reisolated from seedlings killed by the original strains in experiment 62. Typical Rheosporangium, identified by presporangium formation, was easily recovered from the damped-off seedlings in pots of pines only, those of beets only, and the pots in which both hosts were sown. The recovery of a virulent Pythium strain from a single one of the pots inoculated with the weaker Rheosporangium shows that despite the absence of disease in the controls a slight amount of contamination did occur. However, the comparative ease with which the Rheosporangium was isolated from seedlings in other pots inoculated with it and the fact that it has never been obtained in the numerous cultures made from controls and from pots inoculated with other organisms leave little room for doubt that the strains isolated were really recoveries of the Rheosporangium used in the original inoculations. The results of reinoculation with these strains are shown in Table VII.

From Table VII and by comparison with Table VI it appears—

(1) That in one experiment each on jack pine and red pine the reisolated Rheosporangium strains gave positive results. In a second experiment on jack pine (No. 67) the difference between the Rheosporangium pots and the controls was not significant.

(2) That, as in Table VI, the Pythium strains used proved on the whole decidedly more parasitic than the Rheosporangium strains. In experiment 66 this is not shown by the percentage of seedlings damped-off, but is sufficiently evident when the germination loss as well as the subsequent damping-off percentage is considered, the survival being here, as in most other cases in which

either of the groups of pots compared is seriously affected by parasites, the most convenient index of the comparative activity of the fungi used. In such a comparison as that between the Rheosporangium pots and the controls in experiment 68 (Table VII), accidental variations in emergence, of course, overshadow the slight effect of the fungus, and the definitely determinable percentage of loss after emergence is the only value which can serve as a basis for any definite conclusion.

TABLE VII.—*Results of inoculations on pine seedlings with initial and reisolated strains of Rheosporangium aphanidermatus compared with parallel inoculations with Pythium debaryanum.*

Experiment number, host, and inoculating fungus.	Reisolation source.	Number of pots.	Number of strains.	Results.		
				Emerged (per 5-pot unit).	Damping-off (per cent).	Survival (per 5-pot unit).
No. 66, Pinus banksiana:						
Rheosporangium—						
Strain 229	Edson from beet	5	1	63	9	58
Strain 351	do	5	1	80	52	39
Strains 403 and 404	Strain 229 from beet	10	2	78	43	44
Strains 405, 406, and 407	Strain 351 from beet	15	3	63	43	36
Strains 417, 430, and 433	Strain 351 from pine	15	3	72	42	41
Average		50	10	70±2.7	40	42±3.8
Controls		25		75	15	64
Pythium, average		235	47	43	33	29
No. 67, Pinus banksiana:						
Rheosporangium—						
Strain 229	Edson from beet	5	1	107	0	107
Strain 351	do	5	1	88	6	83
Strains 403 and 404	Strain 229 from beet	10	2	91	8	84
Strains 405, 406, and 407	Strain 351 from beet	15	3	92	4	88
Strains 417, 430, and 433	Strain 351 from pine	15	3	83	3	80
Average		50	10	90±2.4	4	86±2.9
Controls		23		87	5	83
Pythium, average		235	47	51	26	38
No. 68, Pinus resinosa:						
Rheosporangium—						
Strain 229	Edson from beet	5	1	105	0	105
Strain 351	do	5	1	124	0	124
Strains 403 and 404	Strain 229 from beet	10	2	102	1	101
Strains 405, 406, and 407	Strain 351 from beet	15	3	105	5	100
Strains 417, 430, and 433	Strain 351 from pine	15	3	100	3	97
Average		50	10	105±2.3	2	102±2.5
Controls		18		104	0	104
Pythium, average		235	47	86	27	63

A frequency graph based on the survivals of the 50 individual pots inoculated with Rheosporangium in experiment 68 yields a rather interesting asymmetrical curve (fig. 17). The shape of the curve is taken as indicating that in a large number of the pots the inoculation produced no effect, while in the smaller number of pots in which the inoculation apparently "took," the loss was rather heavy. This is a rather common phenomenon in inoculations which are only partly successful, part of the pots being free or practically free from loss, while others are nearly cleaned out. It will be seen again in

figure 18. This suggests, further, that part of the lack of activity was due to the failure of the fungus to maintain itself vigorously in the soil till the pines reached a stage of sprouting in which they could be readily attacked. Direct inoculations after the seed starts to sprout are therefore desirable to supplement the experiments reported. The survivals in the controls did not show any such asymmetrical distribution.

While the Rheosporangium has given rather definite evidence of parasitism on *Pinus banksiana* under favorable conditions, the activity of the strains available has been much less than that of the *Pythium debaryanum* strains. In view of the fact that the fungus has not so far been isolated from pine it can be concluded to have no general importance in pine seed beds. Its very rapid growth on prune agar makes it very easy to isolate when present.

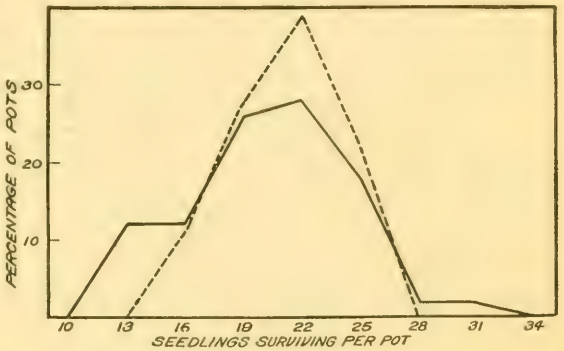

FIG. 17.—Diagram showing the results of inoculation of *Pinus resinosa* seedlings with *Rheosporangium aphanidermatus*, as indicated by the number of seedlings surviving in inoculated pots (solid line) and control pots (broken line). The shape of the curve for the inoculated pots is taken as indicating that a large proportion of them were entirely unaffected by inoculation, while those which were at all affected suffered considerably. This is a frequent result in inoculations with weak parasites added at the time of sowing the seed.

PHYTOPHTHORA SPP.

Phytophthora fagi R. Hartig has been commonly reported as the cause of death of seedlings of various plants in Europe, including conifers and herbaceous plants as well as beech (5, 8, 15, 55, 56, 57, 59, 73, 104.) It has been grouped with the rather indefinite *Phytophthora omnivora* and with *P. cactorum*, the enemy of cactus, ginseng, and other plants. Wilson (147, p. 54) considered it distinct, but Rosenbaum (114), in his biometric comparison of *Phytophthora cactorum* and a single strain of *P. fagi*, failed to find significant morphological differences. If *P. fagi* is even physiologically different from the American strains of *P. cactorum*, its introduction into the United States is to be guarded against. There is certainly no fungus in the United States causing the damage to coniferous seedlings which European reports have attributed to *P. fagi* there. As *P. fagi* attacks roots, it presumably can be carried in soil as well as on plant parts.

A test made on jack pine with a culture of *Phytophthora cactorum*, furnished by the department of plant pathology of Cornell University, resulted negatively. At the time of sowing the seed three pots were inoculated with cultures on nutrient agar inserted at several points in each pot. After emergence additional fragments of prune-agar cultures were placed in contact with the seedlings, and they were

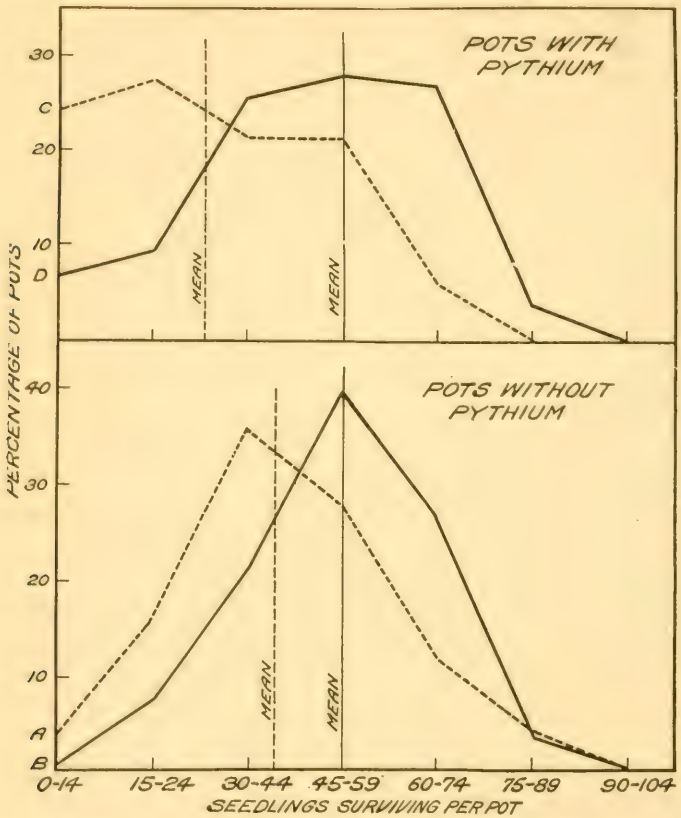

FIG. 18.—Frequency of pots with different numbers of surviving seedlings of *Pinus banksiana*, inoculation experiment No. 31. The solid lines represent pots to which cultures of saprophytic organisms were added. The broken lines are based on pots to which no saprophytes had been added. The solid lines are based on 78 pots in the upper graph and 80 pots in the lower one; the broken lines on 33 pots in the upper and 25 pots in the lower graph. *Pythium debaryanum* was added just after sowing the seed at a single point in each pot represented by the two upper lines. Cultures of saprophytes were applied broadcast two days before the Pythium inoculations were made.

sprayed with a spore suspension. The pots were covered with glass to increase atmospheric moisture, and the seedlings were occasionally sprayed with an atomizer. The soil was an autoclaved mixture in which simultaneous inoculations in a different room with Pythium and Corticium proved successful. The failure of the Phytophthora may possibly have been due to the lower temperature at which the pots inoculated with it were kept (15° to 20° C.).

A species of Phytophthora was isolated by Mr. R. G. Pierce from damped-off *Pinus resinosa* in Minnesota and used in four inoculation experiments, the results of which appear in Table VIII. In the first of these experiments unboiled water was used on the pots, and mice obtained access to the pots of the second test. Probably as a result of these things infection occurred in the controls in both cases, and the results were inconclusive; in the later experiments these two sources of infection were eliminated, and in experiments 68 and 72B the controls were free from disease. Parasitic activity was indicated rather strongly in experiments 68 and 72 (on *P. resinosa* and *P. banksiana*) and to a certain extent in experiment 66. In experiment 67 it was evident that the Phytophthora was nearly or entirely inactive. Comparison of the results in experiments 66 and 68 with the results from inoculations with *Rheosporangium aphanidermatus* in the same experiments (Table VII) suggests that the Phytophthora may be better able to attack the pine from which it was isolated than the Rheosporangium, while the latter fungus caused considerably more destruction to *Pinus banksiana* than the Phytophthora. Comparison of the results in the pots inoculated with Phytophthora and those inoculated with *Pythium debaryanum* in all the experiments indicates that the Phytophthora strains used were less virulent than most of the strains of *P. debaryanum* and very certainly less destructive than the most active strains of either *P. debaryanum* or *Corticium vagum*. This species of Phytophthora has been reisolated from damped-off *Pinus ponderosa* in experiment 72.

Direct inoculations of the stems of seedlings of *Pinus resinosa* soon after they emerge from the soil have so far confirmed the lack of parasitism of *Phytophthora cactorum* and of the cultures of *Phytophthora* sp. grown by the writer. The identity of this species has not yet been determined. It is able to grow only about one-fourth as rapidly as *Pythium debaryanum* on the medium which has been used for isolation and may therefore be more common in the seed beds than the small number of isolations by the planted-plate method would indicate. However, its oospores, larger and darker than those of *Pythium debaryanum* (usually over 20 μ in diameter), should have been recognized in the routine microscopic examination of planted-plate cultures had this species been frequently present, even if it had not grown fast enough to get out ahead of the other organism and allow isolation. It is not believed that it is common enough in pine seed beds to be of importance, even if other strains should be found more virulent than those which have been available.

MISCELLANEOUS PHYCOMYCETES.

A fungus, apparently referable to the somewhat indefinite *Pythium artotrogus* (Mont.) De Bary, was isolated by Mr. Glenn G. Hahn from *Pinus resinosa* in Michigan and from damped-off *Pinus bank-*

siana in pots of autoclaved soil which had received tap water at Washington, D. C. It agreed both in the appearance and measurements of its spiny oogones and smooth oospores with *Pythium artotrogus* (*P. hydnosporus*) as described and figured by Butler (23). In addition to the spores which Butler describes, there appeared in apparently pure prune-agar cultures of different strains bodies with smooth walls, of somewhat irregular ovoid outline, and mostly larger than either oospores or oogones. They are very much less abundant than the sexual spore forms. Their greatest diameter varied from 11 μ to over 40 μ. The germination of these bodies was not observed. Efforts to induce the fungus to produce swarm spores by growing them in liquid nutrient media and transferring them to pure water were unsuccessful. This failure to produce zoospores is further indication of the identity of the fungus with that described by Butler, who says that asexual reproduction is unknown.

The strain from Michigan was a rather weak growing organism, difficult to maintain in tube cultures without rather frequent transfers. Its parasitic activity in the experiments reported in Table VIII is nil or negligible. Because of the poor seed and small number of seedlings involved in experiment 72B, the percentage of damping-off there given means only a single seedling dead. The Washington strains, on the other hand, though evidently not strong parasites, did apparently cause the death of a number of seedlings. The best evidence of this is in experiment 68, in which there was damping-off in each of the five 5-pot units containing the Washington strains and none in any of the 18 control pots. The available strains were less active not only than *Pythium debaryanum*, but less than the Rheosporangium and Phytophthora strains used. The fungus is believed to be a potential parasite on pine seedlings, but not one of any general importance. What is probably the same fungus had appeared in the writer's cultures from western nurseries in conjunction with *P. debaryanum*, but not commonly, and it had not been isolated. While its growth rate is only about half that of *P. debaryanum* on prune agar, it is nevertheless so much faster than that of many fungi that it should have been more often obtained in culture were it at all common in damped-off seedlings.

Another fungus, presumably an oomycete but producing only chlamydospores in the writer's cultures, was obtained from damped-off olive seedlings furnished by Prof. W. T. Horne and from soil direct, both at Berkeley, Calif. The fungus is apparently the same as one which has been occasionally seen in cultures from pine seedlings in the Middle West, but had not before been isolated. The hyphæ are ordinarily nonseptate, and the growth on corn-meal agar is superficially much like that of *Pythium debaryanum*, but with greater tendency toward local zonation and aerial growth and less

than half as rapid. Chlamydospores are mostly intercalary, at first subspherical, soon becoming polygonal, and after a few days they shrivel and exhibit thick, angular walls. In size the unshrunken spores usually lie between 8 and 12 μ in diameter, but bodies as large as 20 μ occasionally occur. Antheridia have not been observed, and the shriveled bodies are not believed to be oospores, though the observations made have not been sufficient to exclude such a possibility. No other spore form was obtained in water culture, using various nutrient substrata. In inoculation the strain from olive (the "undetermined Phycomycete" included in Table VIII) has given negative or nearly negative results in three inoculation tests in which other fungi gave positive results. In a test not included in the table, in which *Pinus ponderosa* was the trial host, damping-off was slightly higher in the inoculated pots than in the controls, but the difference was apparently due to accidental infection with Botrytis and *Pythium debaryanum*. As all the seedlings in pots inoculated with *P. debaryanum* in this additional experiment were killed, the relative unimportance of this strain of the small-spored fungus was further indicated. An additional test of both the olive strain and the strain from soil was made by inoculating seedlings of *Pinus banksiana* and *P. ponderosa* growing on filter paper in Petri dishes. Some of these were kept wet with water, some with an inorganic culture solution, and some with the inorganic solution plus peptone and dextrose. Agar cultures were applied directly to the seedlings. The seedlings inoculated with the small-spored fungus remained alive as long as the control seedlings, while parallel inoculations with *Pythium debaryanum* resulted in the early decay of the seedlings.

TABLE VIII.—*Results of inoculations with miscellaneous oomycetes on pines in autoclaved soil at the time of sowing.*

[In all the experiments included in this table, the inoculum consisted of fragments of agar cultures distributed with the seed at one side of each pot over about one-fourth of the pot area. The controls received sterile agar in the same way.]

Experiment number, host, and inoculating fungus.	Number of pots.	Results.		
		Emerged.	Damping-off.	Survival.
No. 66, Pinus banksiana:		*Per 5-pot unit.*	*Percent.*	*Per 5-pot unit.*
Phytophthora sp.—				
Strain 358	5	70	30	49
Strain 372	5	75	28	54
Strain 375	5	94	16	79
Pythium artotrogus (?), Michigan strain	4	115	14	99
Undetermined Phycomycete	5	83	9	76
Controls	25	75	14	64
No. 67, Pinus banksiana:				
Phytophthora sp.—				
Strain 358	5	96	1	95
Strain 372	5	88	3	85
Strain 375	5	99	0	99
Pythium artotrogus (?), Michigan strain	5	102	2	100
Undetermined Phycomycete	5	98	0	98
Controls	23	87	5	83

TABLE VIII.—*Results of inoculations with miscellaneous oomycetes on pines in autoclaved soil at the time of sowing*—Continued.

Experiment number, host, and inoculating fungus.	Number of pots.	Results.		
		Emerged.	Damping-off.	Survival.
No. 68, Pinus resinosa:		*Per 5-pot unit.*	*Percent.*	*Per 5-pot unit.*
Phytophthora sp.—				
Strain 358	5	104	7	97
Strain 372	5	109	18	89
Strain 375	5	98	5	93
Pythium artotrogus (?), Michigan strain	5	121	0	121
Pythium artotrogus (?), Washington, D. C.—				
Strain 821	5	122	1	121
Strain 823	5	120	9	109
Strain 831	5	96	5	91
Strain 832	5	110	6	103
Strain 833	5	94	1	93
Undetermined Phycomycete	5	84	2	82
Controls	18	104	0	104
No. 72A, Pinus resinosa:		*Per 3-pot unit.*		*Per 3-pot unit.*
Phytophthora sp.—				
Strain 372	3	20	35	13
Pythium artotrogus (?), Michigan strain	3	62	0	62
Pythium artotrogus (?), Washington, D. C.—				
Strain 821	2	45	7	42
Strain 831	3	37	0	37
Strain 833	3	40	25	30
Controls	16	35	5	33
No. 72B, Pinus ponderosa:				
Phytophthora sp.	3	8	50	4
Pythium artotrogus (?), Michigan strain	3	13	8	12
Pythium artotrogus (?), Washington, D. C.—				
Strain 821	3	11	0	11
Strain 831	2	29	6	27
Strain 833	2	6	0	6
Controls	14	9	0	9

OTHER FUNGI.

Data on the possible relation between various other fungi and the damping-off of conifers have been already summarized by Hartley, Merrill, and Rhoads (68, p. 546–550). *Pestalozzia funerea* on the basis of the experiments of Spaulding (135), *Botrytis cinerea* on the basis of observation and very preliminary inoculations, and *Trichoderma koningi* on cultural evidence only are all believed to be potential causes of damping-off, though not ordinarily important. *Alternaria* sp. is under a certain amount of suspicion on account of its frequent association with the damping-off of conifers, but it has never been used in experiments. *Rhizopus nigricans* (incorrectly reported as Mucor), *Trichothecium roseum*, *Rosellinia* sp. from nursery soil, *Chaetomium* sp. from maple roots, strains of Penicillium and Aspergillus, *Phoma betae*, and *Phoma* spp. are all reported to have been used in inoculations with negative results.

Since the publication of the above summary a preliminary successful inoculation experiment with *Botrytis cinerea* on recently emerged *Pseudotsuga taxifolia* has been found briefly mentioned in an article by Tubeuf (140) on another disease. Further experiments with va-

rious strains of Botrytis, both from conifers and from other hosts (the latter supplied by the departments of plant pathology of the California and New York (Cornell) Agricultural Experiment Stations), have already yielded confirmatory evidence of the parasitism of *B. cinerea*.

While a considerable number of fungi have been considered in the foregoing, it is entirely possible that there are still parasites which have received no consideration and that some of them may perhaps be important. The moist-chamber method of culturing parasites for isolation yields only those which produce spores readily; the planted-plate method is not well adapted to the isolation of slow-growing fungi or bacteria. It is suggested that in further culture work with damped-off conifers an attempt be made to secure slow-growing organisms by dilution plates of teased-up fragments of recent lesions.

RELATIVE IMPORTANCE OF THE DAMPING-OFF FUNGI ON CONIFERS.

The relative importance of the different damping-off parasites is something that has not been thoroughly investigated for any host. The most information on this point is that given by Busse, Peters, and Ulrich (22) for sugar beet. In this case they find the specialized *Phoma betae* distinctly the most important, with *Pythium debaryanum* second and *Aphanomyces levis* third.

Peters (100) apparently considered Rhizoctonia unimportant as a cause of beet damping-off. The opposite was indicated by a small number of cultures by Edson (38) from beet seedlings on Kansas and Colorado soil. These yielded more *Corticium vagum* than any other parasite and no Pythium at all. Johnson (81) states that most of the damping-off of tobacco seedlings is due to *Pythium debaryanum* and *Corticium vagum*. Atkinson (1), speaking for cotton in Alabama, and Sherbakoff (127, p. xcv; 128; 129), speaking for truck crops in Florida, make *Corticium vagum* the important damping-off parasite, with *P. debaryanum* negligible. Horne (oral communication) found the same situation in tobacco seed beds in Cuba. Atkinson (3), in an article on trees, held that many of the cases of damping-off attributed to *P. debaryanum* are in reality due to *C. vagum*. Peltier (98, pp. 336–337) has reported *Rhizoctonia solani* as the cause of damping-off of a large number of plants, recording his observation of the damping-off of seedlings of nearly 50 species of miscellaneous genera and cuttings of 13 different species, all of which he attributes to the Rhizoctonia. He does not state whether in this case he used diagnostic methods likely to detect *Pythium debaryanum* if it had been present.

For the conifers, no very reliable data on relative importance have been published. Numerous European reports emphasize the damage due to *Fusarium* spp., while a smaller number attribute loss to *Phytophthora fagi* or to both. There seems to have been little effort to determine the presence or absence of Corticium or Pythium, so these reports can not be given great weight. Spaulding's evident belief (136) in the importance of Fusarium has more weight, as he was on the lookout for the other fungi; the moist-chamber diagnostic method employed in most of this work was, however, not well adapted to the detection of either one. The same is true of the work of Rathbun (106), in which dilution plates of seed-bed soil were employed. Rankin (105) attributes to *Fusarium* spp. the greatest importance in tree seed beds in this country, with *Pythium debaryanum* and *Rhizoctonia* spp. important in certain cases. Gifford (46) emphasizes the importance of Fusarium, while Clinton (28) apparently found Rhizoctonia (*Corticium vagum*) especially prevalent in the examinations he made.

On the basis of the data presented or summarized in this bulletin, it is believed that of the various organisms which have been connected with damping-off in coniferous seed beds *Pythium debaryanum*, *Corticium vagum*, and *Fusarium* spp. include all of importance. The others, either because of low indicated virulence or infrequent occurrence, and in most cases both, do not seem to merit extensive consideration.

In order to form an idea of the relative frequency of the parasites named above as important, there have been brought together in Table IX the results of the examination of 438 damping-off foci in untreated beds and 304 foci which have appeared in beds which had received various disinfectant treatments. The data are presented by foci rather than by individual seedlings, as was done in the census reported by Busse and his coworkers. Most of the diagnoses were made by planting recently diseased seedlings in plates of solidified prune agar, all the seedlings taken from the same focus, or "patch," of damped-off seedlings being put into the same Petri dish. The resulting growth was in some cases transferred to a tube for later examination, but was usually examined directly in the plate. In a smaller number of foci the seedlings were macerated and examined directly without recourse to culture methods. As *Pythium debaryanum* does not commonly fruit in diseased seedlings of pine or of tobacco (81) and its hyphæ are both difficult to find and not in themselves considered a sufficient diagnostic character, this latter method of examination is not so satisfactory for the determination of Pythium as it is for Corticium, which is easily recognized by its

thick-walled truncate-tipped hyphæ and characteristic branching. A further difficulty in the direct-examination method, unless the seedlings are sectioned, is in distinguishing between Corticium hyphæ which are in the tissues and those outside. The well-known habit of the Corticium of sending hyphæ superficially over the surface of plants which it is not appreciably injuring makes it evident that only hyphæ actually found in the tissues have diagnostic value. Direct microscopic examination is, furthermore, very likely to fail to detect Fusarium. The planted-plate method therefore appears the better of the two, and the results of the culture diagnoses appearing in the lowest two lines of Table IX deserve probably more attention than the total occurring a few lines above, in which the results of direct examination of the seedlings are also included. The high proportion of Corticium reported from the Michigan and Minnesota nurseries is probably due in part to the fact that most of the examinations made there were of the direct microscopic type.

TABLE IX.—*Results of the examination of damping-off foci in coniferous seed beds for Pythium debaryanum, Corticium vagum, and Fusarium spp.*

Grouping.	Untreated beds.				Beds of heated soil.				Beds treated with strong acids.			
	Foci examined.	Number showing—			Foci examined.	Number showing—			Foci examined.	Number showing—		
		Pythium.	Corticium.	Fusarium.		Pythium.	Corticium.	Fusarium.		Pythium.	Corticium.	Fusarium.
By locality:												
Berkeley, Calif.	4	3	1	2	----	----	----	----	5	5	0	2
Manitou, Colo.	22	0	19	0	----	----	----	----	----	----	----	----
Monument, Colo.	34	6	20	13	----	----	----	----	----	----	----	----
Garden City, Kans.—												
Garden City Nurseries	18	4	0	9	14	5	0	7	28	8	2	9
Kansas Nurseries (sand)	20	4	9	7	15	2	0	6	16	9	2	8
Halsey, Nebr.	224	124	45	155	28	10	0	17	99	34	2	61
Cass Lake, Minn.	42	15	21	5	----	----	----	----	1	0	0	0
Dundee, Ill.	4	0	4	1	----	----	----	----	----	----	----	----
East Tawas, Mich.—												
Beal Nurseries (sand)	45	14	33	2	----	----	----	----	1	1	0	0
East Tawas Nurseries	13	11	7	1	----	----	----	----	13	7	6	2
Washington greenhouse	12	3	3	9	7	2	0	3	----	----	----	----
Total:												
Number	438	184	162	204	64	19	0	33	163	64	12	82
Percentage	100	42	37	47	100	30	0	52	100	39	5	50
By diagnostic methods:												
Direct examination—												
Number	156	39	108	25	0	----	----	----	16	6	8	4
Percentage	100	25	69	16	----	----	----	----	100	38	50	25
Planted-plate cultures—												
Number	282	145	54	179	64	19	0	33	147	58	4	78
Percentage	100	51	19	63	100	30	0	52	100	39	3	53

TABLE IX.—*Results of the examination of damping-off foci in coniferous seed beds for Pythium debaryanum, Corticium vagum, and Fusarium spp.*—Con.

Grouping.	Beds treated with formaldehyde.				Beds treated with copper sulphate.				Beds treated with zinc chlorid.				All treated beds.			
	Foci examined.	Number showing—			Foci examined.	Number showing—			Foci examined.	Number showing—			Foci examined.	Number showing—		
		Pythium.	Corticium.	Fusarium.		Pythium.	Corticium.	Fusarium.		Pythium.	Corticium.	Fusarium.		Pythium.	Corticium.	Fusarium.
By locality:																
Berkeley, Calif.													5	5	0	2
Manitou, Colo.																
Monument, Colo.																
Garden City, Kans.—																
Garden City Nurseries	6	0	6	3	1	0	0	1	3	1	0	3	52	14	8	23
Kansas Nurseries (sand)													31	11	2	14
Halsey, Nebr.	31	20	1	24	6	3	0	5	8	0	0	5	175	67	3	112
Cass Lake, Minn.													1	0	0	0
Dundee, Ill.																
East Tawas, Mich.—																
Beal Nurseries (sand)													1	1	0	0
East Tawas Nurseries	8	5	4	0	6	5	3	3	5	3	0	2	32	20	13	7
Washington greenhouse													7	2	0	3
Total:																
Number	48	25	11	27	13	8	3	9	16	4	0	10	304	120	26	161
Percentage	100	52	23	56	100	62	23	69	100	25	0	63	100	39	9	53
By diagnostic methods:																
Direct examination—																
Number	8	5	4	0	5	4	2	2	5	3	0	2	34	18	14	8
Percentage	100	63	50	0	100	80	40	40	100	60	0	40	100	53	41	24
Planted-plate cultures—																
Number	40	20	7	27	8	4	1	7	11	1	0	8	270	102	12	153
Percentage	100	50	18	68	100	50	13	88	100	9	0	73	100	38	4	57

The data on the different nurseries do not allow any generalizing on the basis of locality except to say that all of the fungi seem quite generally distributed in the Lake States and Great Plains region. In general, it appears that the Fusaria as a group are more common than either of the other fungi; as they grow more slowly than either the Pythium or the Corticium, they were probably rather more common relatively than even the plate-culture method indicated. It also appears that the Pythium occurred in more foci than the Corticium in the beds examined. Further culture work, perhaps by the method of dilution plates of fragments of lesions, seems desirable, especially in the East and the Northwest, regions in which there are large coniferous nurseries and in which nothing like a parasite census has been attempted. Observations on the type of focus occurring in most of the nurseries in the Rocky Mountains leads the writer to believe that Corticium will be found especially important there.

While the data on the fungi in foci in disinfected beds are insufficient to serve as a basis for much in the way of conclusions for any individual treatment, they in general agree with the assumption, which knowledge of the fungi would favor, that Corticium is the

most easily controlled by soil disinfection (see the bottom line in the last four columns of Table IX). Its poor adaptation for aerial dissemination would lead one to expect to find it seldom in beds treated with efficient disinfectants. The entire absence of Corticium in heated soil therefore seems somewhat significant. The rather high Corticium yield in the formaldehyde plats is of some interest in view of the reported inefficiency of formaldehyde in destroying *Corticium vagum* on potato tubers (48, 50). As will be noted from the data given, more than one suspected parasite was often found in what appeared to be a single focus. This was probably in some cases due to independent foci being nearly concentric; it also in some cases undoubtedly means that one of the organisms found was only secondary. In the beet-seedling cultures by Busse and his associates, individual seedlings yielded two or more potential parasites in 100 of their nearly 1,300 examinations. It not infrequently happened in the work on pine seedlings that no fungus recognized as a likely parasite could be isolated. This was especially true in plate cultures when Rhizopus or Trichoderma happened to be abundant, as both are very fast growing and often suppress parasites. This is an additional reason for the development of some method as a dilution plate of lesion fragments for diagnosing damping-off.

Even an accurate and complete census of the organisms present in the different foci could not be directly interpreted in terms of relative importance. None of the parasites so far used in inoculation have been vigorously parasitic under all conditions. Of both *Corticium vagum* and *Pythium debaryanum* some strains, microscopically indistinguishable from the others, are very weak as parasites. Only part of the Fusarium species are parasitic on pine, and data showing which are and which are not parasitic are known for only a very few. There is therefore no fungus which can be said positively to be the cause of any particular damping-off "patch" simply because it was found in some of the dead seedlings in the patch. In an occasional exceptional case, such as the large Corticium patch in figures 7 and 8, there is such a vigorous growth of the fungus that its predominance is undoubted, but such cases are rather rare. A census throws light on the importance of the different fungi, but can be interpreted only in the light of inoculation results.

For Pythium and Corticium the inoculation data do not permit any simple comparison between the two, for the reason that neither is uniform. Each has strains of high virulence and strains having practically no effect on pines. In the inoculations in autoclaved soil at sowing time the strongest strains of *Corticium vagum* have on the whole caused more damage than any of the Pythium strains, but, on the other hand, there has seemed to be a higher proportion of very

weak strains of *C. vagum* than in the case of Pythium. In inoculations on *Pinus banksiana* and *P. ponderosa* in Kansas sand treated with acid followed by lime, the average Corticium was very much more destructive than even the strongest Pythium strains, allowing practically no germination in most cases. On the other hand, in experiments in which the inoculum was applied directly to *Pinus resinosa* and *P. ponderosa* seedlings, either immediately after germination or after the older parts had become resistant, the Pythium has been the more effective. The inoculation evidence so far available justifies so nearly equal emphasis on the two that it can practically be eliminated from the calculations. It is the writer's opinion that the Corticium strains are probably rather less virulent on the average than the Pythium strains, but perhaps better able to maintain themselves and spread from one seedling to another in most soils. The evidence of Table IX that the Corticium seemed less frequent in the damping-off foci is more or less counterbalanced by the apparent larger size of many of the disease patches which it seems to cause in the seed beds. Nearly all the large clean areas such as are shown in figures 7 and 8 have been found to contain abundant Corticium hyphæ. The evidence on the whole seems to indicate a very nearly equal importance for the two fungi. The Pythium is probably somewhat the more important for the stations at which most of the cultures in Table IX were made, but the Corticium has received more emphasis from other observers in this country and is indicated by the writer's observations to be more important in the western mountains than any other damping-off fungus.

The inoculation evidence for *Fusarium* spp., though less complete than for Corticium and Pythium, is nevertheless rather helpful in indicating their importance rating. None of those so far tested in inoculations at sowing have shown the destructiveness of the average strains of Pythium or of the stronger strains of Corticium; while this is only in part a test of virulence and in part a test of the ability of the fungus to grow saprophytically in the soils used, the indication is that no one Fusarium species is the equal in destructive capacity of either *Corticium vagum* or *Pythium debaryanum*. However, when all of the Fusarium species which occur in the seed beds are considered, the group as a whole may prove quite as important or even more important than either of the other two fungi. The data already at hand rather definitely indicate considerable importance for all three.

DAMPING-OFF FUNGI AS CAUSES OF ROOT-ROT AND LATE DAMPING-OFF.

As has been already stated, root-rot, often with frequent recovery, has been commonly observed in seedlings several weeks old. It has been especially common in the vicinity of old damping-off foci in

which *Corticium vagum* appeared to be the active parasite, but beyond this indication of the causal relation of *C. vagum* it was not known which of the damping-off fungi were able to attack the roots of seedlings too old to be killed by damping-off. To throw light on this point, seedlings of *Pinus ponderosa* and *P. resinosa* grown in autoclaved soil in the greenhouse and approximately 1½ months old were inoculated with different fungi. There had been a certain degree of early damping-off in these pots, but it had apparently ceased before the inoculations were made. The inoculum used consisted of cultures on rice introduced through the drainage holes at the bottoms of the pots. The strains of *Pythium debaryanum* and *Corticium vagum* used were the ones which had given maximum results in earlier inoculation experiments at the time of sowing. The strain of *Fusarium ventricosum* was the only one available, and the *Fusarium moniliforme* strains were all of approximately equal virulence, the three used having given as much evidence of parasitism as any of the strains of this species in the earlier damping-off experiments. Three pots of each pine were inoculated with each strain. Two 3-pot units of each pine were set aside as controls and inoculated with sterile rice. In addition, three pots of each pine were kept in the same bench without the addition of any inoculum, for comparison with the controls with rice. The results of this experiment, taken a month after the inoculations were made, with the seedlings averaging 2½ months old, appear in Table X. The roots of the living seedlings were washed out carefully with water to permit examination.

The results in so far as they indicate root-rot of the oldest seedlings are best shown by the figures in columns 4 and 5. These seedlings were so far advanced that the fungi had not been able to kill them, and nearly all would probably have recovered if they had not been dug up. It will be noted from column 4 of Table X that a considerable portion of the *Pinus ponderosa* seedlings with root-rot had already made their recovery apparent by pushing out adventitious roots above the decayed portion at the time they were examined.

For *Fusarium ventricosum* there was only the merest indication of ability to attack pine roots at this stage. For *F. moniliforme* the evidence is somewhat better, more pots being included and the difference in healthy-topped seedlings with injured roots between the inoculated pots and the controls being approximately twice its indicated probable error for each species. The percentage of root-injured seedlings in the *Pythium debaryanum* pots exceeded that in the controls in each species by between three and four times the probable error of the difference, while the difference in percentage between the *Corticium vagum* pots and the controls is approximately four times its probable error in the case of *Pinus ponderosa* and five and one-half times its probable error in the *Pinus resinosa* pots. The

weak point in the results is, of course, the insufficiency of the 6-pot and 9-pot groups as bases for probable-error determination. The indicated relative ability of these different fungi to cause root-rot is about the same as their relative ability to cause the damping-off of sprouting seed and young seedlings, as indicated by the results of the earlier experiments in which inoculations were made at the time of sowing. The fact that only the very strongest available strains were used and that the pots were rather heavily inoculated is to be kept in mind in considering these results. As in the seedlings examined in the nursery beds, when a root system was partly rotted it was only the younger portions of the roots that were affected. The evidence obtained from this experiment needs to be amplified by experiments with other coniferous hosts, other strains of the fungi, and under other conditions. The experiment just described furnishes the only evidence available on the relation of the important fungi *Pythium debaryanum* and *Corticium vagum* to the root-rot of conifers and is therefore presented as a preliminary contribution.

TABLE X.—*Results of root inoculations of older pine seedlings with damping-off fungi.*

Host and inoculating fungus.	Number of—		Seedlings which developed root-rot (per cent).				
			Tops still healthy.				
	Pots.	Seedlings.	Root recovery.		Average of individual pots.	Killed.	Total.
			Started.	Not started.			
1	2	3	4	5	6	7	8
Pinus ponderosa:							
Pythium debaryanum, strains 295, 550, and 810.[a]	9	71	27	25	53±4.5	4	56
Corticium vagum, strains 147, 213, and 747.[a]	9	56	16	34	51±3.5	4	54
Fusarium moniliforme, strains 249, 251, and 260.[b]	9	64	19	27	42±6.2	0	45
Fusarium ventricosum	3	18	17	39	50	0	56
Controls	6	41	2	15	22±6.5	0	17
Controls without rice	3	18	0	17	23	0	17
Pinus resinosa:							
Pythium debaryanum	9	140	3	16	18±4.0	12	31
Corticium vagum	9	146	3	16	21±2.4	13	33
Fusarium moniliforme	8	128	0	11	12±3.7	2	13
Fusarium ventricosum	3	39	0	5	4	0	5
Controls	6	115	0	3	4±2.0	6	10
Controls without rice	3	51	0	0	0	2	2

[a] For relative virulence of these strains on younger seedlings as compared with other strains of the same species, note their position in figures 11 and 14.

[b] For performance of these strains in inoculations at time of sowing, see an earlier publication (68, table 2).

The figures in column 7 give information as to the percentage of late damping-off resulting from the inoculations. A certain percentage of the early type of damping-off appeared in some of the

pots, as there were still present a number of soft-stemmed seedlings from seeds which were slow in germinating. These younger seedlings were excluded in counting the dead, the rule being to include only plants which had developed a sufficiently rigid stem to remain upright after death. Comparison of the percentage of killed with the total percentage attacked for the two pines is rather interesting. As has already been pointed out, while *Pinus resinosa* suffers very heavy damping-off losses at a number of nurseries it seems to be less susceptible than some other species to parasitic injury during the sprouting period, before the seedlings appear above the soil surface. Observation of beds of this species during different seasons has indicated that it has not a greater susceptibility, but rather the fact that its susceptibility lasts longer, which causes it to suffer as seriously as it does at certain nurseries. It is indicated in Table X that the succulent root tips of *Pinus ponderosa* are just as easily attacked by damping-off parasites as those of *P. resinosa*—in fact, considerably more easily attacked, as indicated by the figures in column 8. With the *P. ponderosa* seedlings, however, the older parts of the roots had become resistant at this age in nearly all cases, while of the affected *P. resinosa* seedlings more than one-third were still unable to limit the lesions, and death resulted.

In general, this experiment indicates that *Corticium vagum* and *Pythium debaryanum* are able to cause the death of some pine seedlings which have developed rigid stems and that both are also able, as has been found by other workers in the case of sugar beets, to cause "root sickness," the rot of the younger portions of the root systems, in seedlings which have developed too much resistance to be killed. The evidence for the parasitism of the two Fusarium species on these older root systems is not so good; as in the experiments on younger seedlings, their ability to attack the pines is probably less than that of the other two fungi. Further inoculation experiments are desirable both with these fungi and with others on the roots of seedlings too old to succumb to the more ordinary types of damping-off.

RELATION OF ENVIRONMENTAL FACTORS TO DAMPING-OFF.

In the earlier section dealing with disease control, mention was made of the general belief on the part of men who have had experience with seedling diseases that damping-off is favored by thick seeding, by much organic matter, especially by poorly rotted manure in the soil, and by excessive moisture in the air and soil. It is also commonly stated that high temperature favors the disease; on this point there is perhaps a less general agreement. Practically all the evidence on these points is observational.

DENSITY OF SOWING.

The relation between the disease and thick sowing was strikingly indicated for tobacco seedlings in a single experiment by Johnson (82). For pines the only available information is from four experiments on *Pinus banksiana*. The results of the first two appear in figure 19. In both experiments there is an indication of an increase in the percentage of diseased plants as the seed density is increased. There is, however, no such marked relation as in Johnson's work. As the pines were sown in drills, they were so close together even in the less dense plats that no very great increase in the ease of spread of the disease was to be expected from increasing the density. Greater differences should be expected in broadcast beds. That heavier losses have been found in drill-sown beds than in those sown broadcast (69, 139) is presumably explained by the fact that with equal numbers of seed per square foot of seed bed the seedlings are much closer together in drills than in broadcast beds, and thus the spread of the mycelium of parasites from one seedling to another is facilitated.

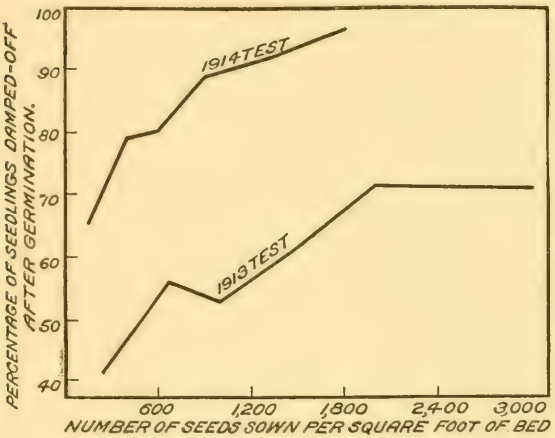

FIG. 19.—Diagram showing the extent of damping-off in drill-sown *Pinus banksiana* in plats with different seed densities. The regular seed density at this nursery was 600 seeds per square foot.

Two tests of different seed densities were also made in 3-inch pots of autoclaved soil in the greenhouse. Each regular pot was sown with 28 seeds (equivalent to 600 per square foot). The pots were inoculated by adding to each a single small fragment of an agar culture of *Pythium debaryanum*. Uninoculated pots showed an emergence of approximately 50 per cent of the seed and were entirely free from subsequent damping-off in both experiments. The results appear in Table XI.

In this case not only the damping-off after emergence but the loss before the seedlings appeared bore an apparent relation to sowing density. In the field experiments there was no evidence that the loss before the seedlings appeared was affected by seed density.

TABLE XI.—*Results of inoculation, at the time of sowing, with Pythium debaryanum on Pinus banksiana in different sowing densities in pots of autoclaved soil.*

[The percentages of "Damping-off," columns 4 and 7, are based on the number of seedlings; the percentages given in columns 3, 5, 6, and 8 are based on the number of seeds.]

Density of seed sowing.	Number of pots in experiment.	Results (per cent).					
		Experiment 58.			Experiment 59.		
		Emerged.	Damping-off.	Survival.	Emerged.	Damping-off.	Survival.
1	2	3	4	5	6	7	8
Regular	10	15	43	10	26	13	23
Double	5	8	65	3	8	91	1
Triple	5	1	100	0	11	34	7
Regular, but 10 additional seeds near point of inoculation	5	6	33	4	17	37	11

MOISTURE AND TEMPERATURE FACTORS.

The relation of damping-off to moisture and temperature are subjects less easily studied. In 1907 and 1908 Mr. W. H. Mast, then supervisor of the Nebraska National Forest, conducted daily counts of the number of seedlings damped-off and compared these records with temperature and rainfall records. The writer in 1909 repeated his work, maintaining parallel records of damping-off, air and soil temperatures, soil moisture, atmospheric humidity, wind movements, and evaporation. The 1909 records of damping-off, temperature, soil moisture, and evaporation appear in figure 20. The damped-off seedlings were counted and removed in the morning and evening, the day period thus being in most cases 10 to 11 hours and the night period 13 to 14 hours. Because the period was not always the same length, the data are reduced to a per hour basis. Air temperature was recorded by a sheltered thermograph 3 feet above the soil surface. The evaporation graph represents the mean loss per hour from two porous cup atometers of the writer's own design, in which the rather long and slender Chamberlain filter bougie was used and supported in a horizontal position just above the soil surface so as to be under as nearly as possible the same atmospheric conditions as the seedlings. The two bougies were placed at right angles to each other in order to eliminate as far as possible the effect of change of wind direction on their mean loss. While the rain-correction mounting had not at that time come into use, the error due to rain absorption appeared negligible; atometers filled shortly before rainfall were read immediately after without any gain being found in the water in the reservoir. The psychrograph and wind-movement records are not presented, as the evaporation values are more easily interpreted. Soil moisture was periodically determined in the soil of the

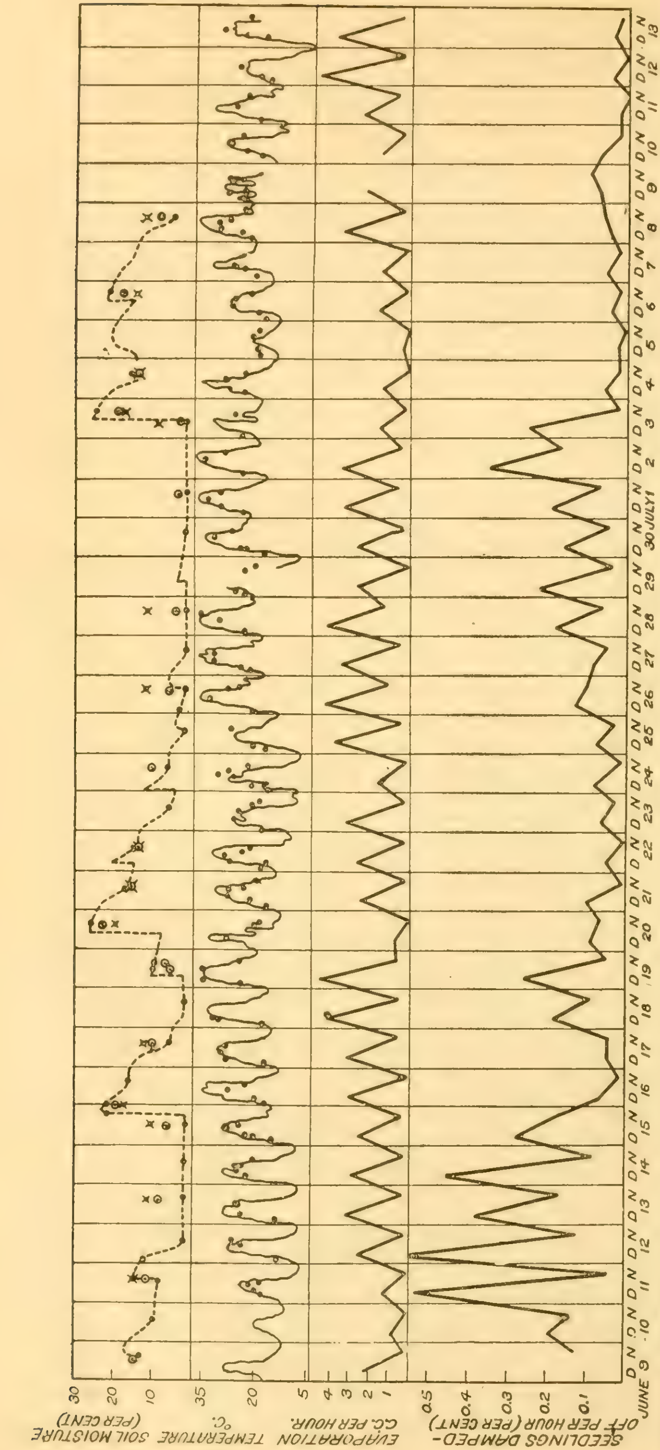

FIG. 20.—Diagram showing the progress of damping-off in beds of *Pinus banksiana* at a nursery in Nebraska and indicating the moisture and temperature conditions. The evaporation from porous-cup atmometers was measured and the dead seedlings counted, night and morning. As the day (D) and night (N) periods into which the time was thus divided were not of equal length, these figures are converted into an hourly basis. The temperature curve is taken from a sheltered thermograph 1 meter above the surface of the beds, while the dots accompanying this curve indicate readings of a thermometer whose bulb was buried 3 cm. below the soil surface. For the temperature and soil-moisture records, the vertical lines indicate 6.00 a. m. Explanation of symbols referring to soil moisture: ⊙=At depth of 0 to 0.6 cm., ⊙=at depth of 0 to 2.5 cm., ×=at depth of 2.5 to 5 cm.

plats on which the seedling counts were conducted, each determination representing two, and in some cases four, points. The determinations for the upper one-fourth inch of soil, made more frequently than for lower levels, are connected in figure 20 by a dotted line, which gives some idea of the amount of moisture in the surface soil during the periods between determinations. The determinations were too infrequent to permit anything more than an estimate of the moisture conditions between determinations, but the writer, having before him the records of the times and amounts of rainfall and artificial watering as well as the evaporation and soil-moisture determinations, is in a better position to make such an estimate than the reader. The dotted line which gives this estimate should not be depended on to show what the percentage of moisture was at any one time, but is believed reasonably reliable as showing whether in general the soil was wet or dry. In interpreting the soil-moisture records, it should be kept in mind that the soil was very sandy, the wilting coefficient of composite samples from various parts of the nursery, as determined by the indirect method of Briggs and Shantz in the Laboratory of Biophysical Investigations of the Bureau of Plant Industry, being only 3.4 per cent. The hygroscopic moisture in dry air for the soil of the plats actually under consideration was indicated by repeated determinations for the surface soil on dry days to be in the neighborhood of or slightly below 2 per cent. The nursery is located in a region of large temperature fluctuations, where the air during the day is generally dry, and consequently the dew is heavy at night.

The first result of interest is the difference between the damping-off for the day and the night periods. In the records of every day but two, more seedlings went down during the day period than during the night, the differences in most cases being large. As the evaporation and temperature showed similar day and night fluctuations, it is difficult to say whether temperature or moisture conditions were responsible. The other interesting result brought out by the graphs is the sudden drop in the general level of the damping-off graph following the rains of June 15, June 19–20, and July 3. In each of these three cases the damping-off came up again only after the soil moisture came down.

The fact that in the daily fluctuations the damping-off varied directly with the evaporation rather than inversely is an apparent contradiction of the generally accepted doctrine that moisture favors the disease. This contradiction is, however, only apparent. During the first part of the damping-off period, when the seedlings are still soft, the recognition of damping-off depends on the decay of that part of the stem just above the soil surface which allows the seedling to fall over. This usually takes place at this nursery as

a result of the extension upward of lesions which have started on the parts a little below the soil surface. It is supposed that such decay takes place most rapidly at high temperatures and that it is the temperature rather than the evaporation graph which the damping-off is following in these early day and night fluctuations. During the latter part of the damping-off period a dying seedling shows its first signs of distress in the drying up of its leaves, the stem being too stiff to go down until after the infection has gone far enough in the roots to cut off most of the water supply. It is, of course, under dry conditions that such a sign of distress will be most in evidence. During the latter part of the damping-off period it is therefore altogether likely that the day and night fluctuations are caused, at least in part, by the higher evaporation rate which obtains during the day. This is a relation not to the rate of progress of the disease, but rather *to the rate at which the symptoms of disease appear in plants already seriously affected.*

The drop in damping-off following the increased soil moisture of June 15, 19–20, and July 3 also apparently contradicts established doctrine. While it is ordinarily true that a wet soil is a cold soil and that in the rainy weather which causes wet soil the evaporation is usually low, it does not seem possible on inspection of the graphs for these items to attribute entirely the reduction of damping-off during these periods of wet soil either to low temperature or to low evaporating power of the air. Lowered soil temperature probably had something to do with the reduced loss following the rains. It is also suggested that a sudden change in moisture content may temporarily hinder a soil fungus by decreasing its air supply. In this sandy soil the fungi can work at very considerable depths during dry periods. Initial lesions have been found as much as 12 inches below the surface. If this soil is as completely changed in its aeration qualities by wetting as the sandy soil with which Buckingham (19) worked, a rain might result in a rather sudden change in the level at which the fungus is able to operate.

On the whole, the graphs tend to confirm the common statement that high temperature favors damping-off. It must, however, be borne in mind that in uncontrolled field plats several factors vary simultaneously, and it is impossible to definitely attribute any observed phenomenon to any one of them. Furthermore, it is not possible to say for the seedlings at different ages just how long it will take a factor to exert an effect on the damping-off curve. An additional consideration is that a method of investigation which gives entirely reliable information on the speed with which the disease develops does not necessarily throw light on the conditions under which the greatest total amount of disease can be expected before the seedlings become old enough to resist attack. High temperatures,

within reasonable limits, are expected to increase the speed with which the disease works, but these should also hasten the development of the host to a point at which infections are unable to cause death. It is the total amount of damage in the beds rather than the damage per unit of time which is of practical importance. For a number of reasons, then, the method followed in obtaining the data for these graphs can not give information of maximum value. While data of the sort mentioned are of undoubted interest and would be of still more value if the records had been commenced when the first seedlings appeared instead of a few days later, the relation of any specific factor to the total extent of the disease can be better determined by comparing plats in series in which the factors are as far as possible controlled and varied one at a time. To vary soil moisture and soil temperature independently will prove somewhat difficult, but it can be done with the proper facilities. Some work with environmental factors should be done under conditions of artificial inoculation in the greenhouse, in which the different damping-off parasites can be experimented with separately, as it is obvious that the factors which favor the activity of one may not be favorable for another.

CHEMICAL FACTORS.

Chemical factors are presumably also important, as the soil is in most cases the culture medium for both the parasite and the host. The much greater activity of *Pythium debaryanum* in autoclaved soil than in untreated soil may be due to the larger quantity of soluble organic matter commonly present in autoclaved soil. *Pythium debaryanum* has been found more sensitive to unfavorable substrata in artificial culture than *Corticium vagum* and is apparently more dependent on soil organic matter in the nurseries than is *C. vagum*. For example, in the normal humus-containing surface sand in the beds at Cass Lake, Minn., both Pythium and Corticium occurred frequently in the damped-off seedlings, while in beds a few feet distant, from which enough of the surface soil had been removed to leave no humus, nearly all the damping-off foci contained abundant Corticium, and no Pythium could be found. With both fungi and, in addition, with two species of Fusarium (68) heavy inoculation has been more successful in experiments at the time of sowing than light inoculation. This has been thought possibly due in part to the larger amount of nutrient substratum added in the heavy inoculations, allowing better saprophytic development of the fungus in the soil. In each of the two experiments with Pythium reported in Table XI, a 5-pot unit was treated with corn-meal infusion and another with prune infusion at the time of inoculation. In both experiments germination was lower, damping-off after germination higher, and the survival less than half as great in the pots with

infusion as in the inoculated pots not so treated. In the first experiment 5-pot units of unheated soil were also inoculated in the same way. In these also both the units which received infusions showed less germination and more loss after germination than the unit which received no infusion, though the differences were smaller than in the autoclaved soil. In the second experiment the light inoculation used failed to cause material loss in the unheated soil units, even though two of them were treated with the infusion as in the previous test and two others received triple portions of the infusion.

The experience in the nurseries, in which heavy applications of manure, and especially poorly rotted manure, in a number of cases have apparently resulted in increased disease, and the finding of Fred (43) that green manures recently plowed under favored the work of Corticium have already been mentioned. The addition of dried blood at two nurseries in Kansas was in both cases followed by very much heavier loss than in the controlled plats. The only instances known to the writer in which the addition of organic matter to the soil has shown any indication of materially decreasing damping-off (with the exception, of course, of the organic disinfectants) are the result reported by Gifford (46) with tankage, a single case in the writer's experience with bone meal, and the cases in which cane sugar has seemed to decrease losses somewhat (67). It is of some interest to note that the experience available also indicates increased disease as a result of the addition of inorganic nitrogenous substances. Sodium nitrate and sodium nitrite have both given some indication of increasing damping-off. Ammonium sulphate in six separate series has in every case resulted in decreased stands, though unfortunately in experiments in which the damped-off seedlings were not counted. Ammonium hydroxid, though apparently having some initial value as a disinfectant, as indicated by early damping-off losses, in a number of cases has been followed by very heavy total losses. This experience is of some interest in view of the apparently rather general belief that plants on a soil rich in nitrogen are especially susceptible to disease.

The chemical factor for which there is perhaps the most evidence of a relation to damping-off of conifers is acidity. The fact that sulphuric-acid soil treatment has been found to be one of the most effective means of controlling the disease, that its value is mainly lost if lime is later added to the soil, that soil treatment with sulphur in a number of cases has seemed to decrease the disease, and that lime alone and wood ashes have had either no effect or have apparently increased the damping-off whenever they have been tried, all suggest that soil acidity is not favorable to the disease. Additional indication of this appears in figure 12. The acidity determinations serving as the basis for the graph were made by Dr. L. J. Gillespie,

of the Bureau of Plant Industry. The estimates of the relative seriousness of damping-off are very approximate, based in part on observation only. The stations at which damping-off is rated as 1 are places at which it has been reported by nurserymen or foresters as negligible or absent. The estimates for stations 10, 11, 14, and 15 are based entirely on the reports of others, and for station 5 on the basis of counts of damped-off seedlings made by Mr. R. G. Pierce and Mr. Glenn G. Hahn. The writer personally has made the estimates or checked the estimates of the nurserymen at the other stations. A considerable degree of correlation between the hydrogen-ion exponent and the amount of damping-off appears on the face of the graphs, the coefficient being 0.75 ± 0.07. If the correlation is calculated with the H^+ concentration itself instead of its negative exponent, the coefficient, in this case itself negative, is not so high (-0.58 ± 0.11). All of the above data on acidity relation have been picked up incidentally in connection with other work and are merely suggestive. The suggestion, however, seems sufficiently strong to warrant further experimental work directed specifically at the relation between soil acidity and the disease.

The indication in the graph that damping-off is not serious in soils in which the hydrogen-ion exponent (P_H)[6] is less than 6 is of particular interest, in view of the experience of Hawkins and Harvey (71) with cultures of *Pythium debaryanum* on potato juice. They obtained good growth through a range of acidity from P_H 3.4 to 5.8, with no growth or practically none at 3.06 or 8.4. If this represents the acid tolerance of the fungus in the soil solution, it is evident that ordinarily acid soils can not be expected to remain free from damping-off because of inhibition of this particular fungus. This suggests that the apparently salutary influence of soil acidity in decreasing the damping-off of some of the conifers may be exerted in the direction of increasing the resistance of the host rather than of inhibiting the parasites. In any case, it must be kept in mind that as the numerous conifer hosts commonly grown in nurseries have many different habitat preferences and many very different parasites of potential importance, it is not to be expected that there will be found any such constant relation between any factor and the amount of disease as would be expected in a disease in which only a single parasite and a single host are involved.

[6] P_H 6 is equivalent to a hydrogen-ion concentration, expressed in mols per liter, of 1×10^{-6} or 0.000001. The higher the exponent, the smaller the hydrogen-ion concentration. An exponent of 7 means approximate neutrality. In dealing with this exponential form of expression, it should be kept in mind that P_H6 means ten times and P_H5 one hundred times the hydrogen-iron concentration indicated by P_H7. Conversely, the concentration of hydroxyl-ions at P_H7 is one hundred times as great as at P_H5.

BIOLOGIC FACTORS.

Mention has already been made of two strictly biologic factors which may influence the amount of damping-off. Taylor (138) and Rathbun (106) have found Fusarium not only at considerable depths in the soil of pine seed beds, but viable Fusarium spores without hyphæ in the alimentary canals of earthworms and insect larvæ in the soil, and they attribute to the migrations of these and to the tunnels which various animal forms make in the soil a possible importance in the distribution of damping-off Fusaria. A likely relation between *Corticium vagum* epidemics in pine seed beds and the character of the weed flora has also been considered (66).

The relation between the damping-off parasites and other microorganisms in the soil is also a matter of some interest. The effect of the microfauna of the soil on the microflora in general has been considered by Russell and others in a number of papers. The effect of soil disinfection by heat in favoring the work of artificially introduced soil-inhabiting fungous parasites, apparently a rather frequent phenomenon and quite evident in the inoculation experiments with *Pythium debaryanum* reported in the present bulletin, has been in other cases attributed to the removal of bacteria and other fungi which might compete with the parasites (36, 80). Heating soil is known to produce physical changes and also very considerable chemical changes both in organic and inorganic substances. These must not be ignored in considering the effect of previous soil heating on parasite activity. With a view to determining whether all the difference noted in the behavior of *P. debaryanum* in heated soil is due to the direct effects of the heating or in part to the elimination of competing microorganisms, an experiment was conducted in 3-inch pots of autoclaved soil in which 111 of them were inoculated with agar cultures of the Pythium at one point in each pot shortly after seed sowing. The seeds sown in each pot approximated 136, considerably more than are used on equal areas of nursery seed bed. Of these, fifteen 5-pot units and one 3-pot unit had been inoculated broadcast with rice or nutrient agar cultures of various organisms supposed to be saprophytic on pines. These included *Phoma betæ*, *Phoma* sp., *Chætomium* sp. (from a maple root), *Rhizopus nigricans*, *Trichothecium roseum*, *Trichoderma koningi*, *Aspergillus* spp. (including one with black and one with bright-colored spore heads), *Rosellinia* sp. (from soil), *Penicillium* sp., an undetermined bacterium, and three undetermined higher fungi. The whole 78 pots inoculated with *P. debaryanum* and saprophytes, the percentages being based on the total number of seeds in the case of emergence and survival and on the number of seedlings which appeared above ground for damping-off loss, as compared with those which had received the parasite only, gave results as follows:

Pots with saprophytes: Emergence, 47.4±0.86 per cent; damping-off, 9.1 per cent; survival, 41.0±1.23 per cent.

Pots without saprophytes: Emergence, 35.7 per cent; damping-off, 14.3 per cent; survival, 29.2 per cent.

It has been noted in the attempts to diagnose damping-off by planted-plate cultures that when Rhizopus appears *Pythium debaryanum* is not frequently obtained. It is therefore of some interest to note that in this case, in the two 5-pot units which received Rhizopus in addition, the parasite killed only 1.2 per cent and 3.3 per cent, respectively, of the seedlings which appeared above the soil.

At the same time pots not inoculated with parasites were sown, 16 other 5-pot units were inoculated with the same saprophytes as those used in the Pythium inoculated pots, while 25 pots were left entirely without inoculation. A certain amount of damping-off occurred in these pots also as a result of accidental infection. The results were as follows:

Pots with saprophytes: Emergence, 47.8±0.8 per cent; damping-off, 3.9 per cent; survival, 43.7±0.95 per cent.

Pots without saprophytes: Emergence, 43.0 per cent; damping-off, 5.2 per cent; survival, 38.4 per cent.

It is of some interest to note that in these pots also the 5-pot units inoculated with Rhizopus suffered less from damping-off than the average of the saprophyte-inoculated pots.

The probable-error values given above are based on the variability of the emergence and survival figures of the different 5-pot units. No individual figures are available to serve as a basis for the determination of the variability of the pots without saprophytes. The 16 units which support the error determinations are, of course, not a sufficient number to give an entirely reliable index of variability, though these 16 units are respectively derived from the combination of a total of 78 and 80 ultimate units. The distribution of the data appears to be such as to justify the use of probability methods. Of the 64 items which went into the germination and survival calculations, 34 showed a deviation equal to E_s (probable error of a single unit), 9 to a deviation equal to $2E_s$, and only one a deviation equal to $3E_s$.

All of the above figures are based on the results at the end of 10 days after average emergence in the pots. The pots were kept on the benches till practically all damping-off had ceased, 36 days after emergence. As additional accidental infection with saprophytes certainly, and probably with parasites, occurred during this period, the results at the end of the tenth day are considered to give a better indication of the effect of the original inoculations. It is of some interest, however, to note that during the period from the tenth to the thirty-sixth day the difference between the pots to which sapro-

phytes had been added and those which received no saprophytes showed a slight increase, proportionally as well as in the absolute figures. At the end of the 36 days the survivals on all the pots were counted separately. The average number of seedlings per pot were as follows:

Without Pythium:
Without saprophytes, 42.8±2.3; with saprophytes, 52.1±1.1
With Pythium:
Without saprophytes, 30.1±2.4; with saprophytes, 48.1±1.4.

The difference in the survivals in favor of the pots with saprophytes in the first case is 9.3±2.5, three and two-thirds times its probable error. In the second case it is 18.0±2.8, more than six times its probable error.

In general, it appears that in this experiment the inoculation of sterilized soil with saprophytes gave the seedlings some protection both against damping-off due to accidental infection with unidentified parasites and from the additional loss caused by light inoculation with *Pythium debaryanum*. The indication is, as would be expected, that only part of the favoring influence of heat sterilization of soil on introduced *P. debaryanum* is immediately due to the elimination of competition with other fungi. If a mixture of different bacteria and fungi had been added to each of the pots instead of but one or two organisms to each 5-pot unit, the effect might have been more marked.

It will be noted that for all the groups (fig. 18), whether with or without Pythium inoculation and with or without added parasites, the frequency polygon is asymmetrical, indicating by its shape, as did the frequency polygon of survivals in pots inoculated with Rheosporangium (fig. 17), that with infections which do not kill all of the seedlings the selection tends to be by pots rather than by seedlings. In other words, in pots in which the parasites succeed in killing any of the seedlings, they usually kill a considerable number. The tendency is illustrated not only by inspection of the graphs, but by the variability of the different groups. The greater variability in survivals between different pots was in both cases in the groups in which both the damping-off after emergence and the survival percentages indicated the largest loss. The percentages of seedlings damped-off during the entire 36 days following emergence and the coefficients of variability of the survivals of the individual pots at the end of that time are as follows:

Without Pythium:
Without saprophytes, 15.5 per cent damped-off; coefficient of variability, 39±4.2 per cent.
With saprophytes, 11.1 per cent damped-off; coefficient of variability, 28±1.6 per cent.

With Pythium:

Without saprophytes, 27.5 per cent damped-off; coefficient of variability, 67±7.8 per cent.

With saprophytes, 16.9 per cent damped-off; coefficient of variability, 39±2.4 per cent.

This tendency has been frequently observed in experiments in which inoculum is applied to the soil at the time of sowing. Even in experiments in which a relatively small proportion of the seedlings are killed, some of the pots are nearly or entirely cleaned out. It is taken as an indication that failure of inoculation to give results is often due to the inability of the fungus to maintain itself in a vigorous condition till the germinating seed is far enough along to allow easy infection. It may also be in part due to lack of uniformity of the soil in different pots affecting virulence of parasites or resistance of hosts.

In addition to this experiment on autoclaved soil, a somewhat similar experiment was conducted in a nursery in the Kansas sand hills on soil which had been treated with sulphuric acid, followed by lime raked into the soil. Saprophytes, for the most part the same strains that had been used in the experiment in the greenhouse, were added to 24 plats, each of one-half square foot, of *Pinus banksiana* and 24 of *P. ponderosa*, with 16 interspersed plats of each species serving as controls. The saprophytes were growing on rice, part of which was added to the plat with the inoculum in addition to the fungous mycelium. Damping-off was rather heavy in this soil from accidental infection or from parasites which survived the initial acid treatment, no parasites having been artificially introduced. The loss was probably due to *Corticium vagum* or *Fusarium* spp. rather than to *Pythium debaryanum* in this case. In both pines, emergence was slightly better in the control plats than in those to which the saprophytes had been added, the difference for *Pinus banksiana* being less than half its probable error and for *P. ponderosa* slightly more than its probable error. Damping-off for the first few days after emergence was somewhat less in the controls in one species, but higher in the saprophyte-inoculated pots in the other. The saprophytes therefore gave no evidence of effective competition with the parasites on this acid-lime treated sand.

While the competition for water which seems to be the form of competition most common among green vascular plants is not likely to be of significance between fungi such as those which cause damping-off, a very little observation of the growth of mixed cultures of the parasites and other organisms in Petri dishes is sufficient to make one realize that the latter may very considerably decrease the activity of certain of the parasites. In nutrient agar most of the fungi and bacteria introduced from the soil in attempting parasite isolations,

as well as to a less extent the paramecia, nematodes, and amœbæ which develop in such plates, exert a very considerable limiting influence on the growth of most of the damping-off fungi. That they should also limit the growth of parasites in soil, whether by the production of toxic compounds, the exhaustion of food materials, or in other ways, seems entirely reasonable. The results in the writer's experiments on heated soil warrant the suggestion that further trials should be made of the introduction of vigorously growing bacteria or molds, preferably mixed cultures containing a number of different organisms, on seed beds which have been disinfected by some such method as steam or hot water, which leaves the soil in a favorable condition for the development of accidentally reintroduced parasites. If such treatment should be successful in improving the rather disappointing results with soil heating at some nurseries, it might easily become of practical value, as the cultivation of certain of the more easily grown saprophytes on a scale large enough to yield considerable quantities of bacterial or spore suspensions should be fairly easy and entirely practicable in an operation as intensive as that of raising coniferous seedlings.

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SUMMARY.

(1) Damping-off in nurseries is caused mainly by seedling parasites which are not specialized as to host; *Pythium debaryanum* and *Corticium vagum* are probably the most important of these. Damping-off of various herbaceous hosts, including ferns, is often caused by specialized parasites which are limited to a particular host or group of hosts. *Phoma betae* is a rather extreme example of such specialization. For the conifers all the damping-off appears to be due to parasites of the generalized type.

(2) Damping-off of trees, as of herbaceous plants (with the exception of the cases caused by specialized seed-carried parasites), is ordinarily serious only in seed beds or cutting beds in which large numbers of plants are crowded together in a small space. In most of the forest nurseries it is a much more serious matter in conifers than in dicotyledonous seedlings.

(3) The most serious losses in conifers are ordinarily from the root-rot type of damping-off, occurring soon after the seedlings appear above ground and while the hypocotyls are still soft. Losses due to the killing of dormant or sprouting seed by parasites before the seedlings appear above the soil are also frequently serious, sometimes necessarily more so than the later types, as in extreme cases more than half of the seed or young seedlings are destroyed in this way. Damping-off due to infections of parts above the soil surface is serious only under extremely moist atmospheric conditions. The late type of damping-off, in which the roots are rotted after the stem becomes too rigid to be easily decayed, is ordinarily less important than the early types. Seedlings more than 2 months old are ordinarily able to recover from infections by the damping-off fungi. Even after the first month, seedlings with part of their root system killed often recover.

(4) It is possible that damping-off has a certain value as a selective agent by eliminating weak individuals in the seed-bed stage and allowing only the best trees to go into forest plantations. This value, however, is believed to be slight. Disinfectant treatments of seed beds, even when controlling early parasitic losses very well, allow a considerable percentage of disease during the last part of the damping-off period, often as much as occurs at the same stage of development in untreated beds. As it is only this late damping-off in which differences in individual resistance of the seedlings seem to be of importance in determining whether or not they succumb, it is believed that whatever selective value the disease may have will appear in a larger proportion of the damping-off in the treated than in the untreated beds.

(5) Of the different conifers, reports are available as to the susceptibility of 63 species. Species which are especially susceptible at some nurseries may prove more resistant than the average at others. *Pinus resinosa*, which is especially subject to loss at some nurseries, is believed to be so because its growth at these nurseries is slow and its period of susceptibility is therefore especially long. In its early stages it does not seem especially susceptible. Representatives of all the commonly grown genera of the Abietoideæ have been reported by one observer or another as decidedly susceptible. The reports on junipers and other members of the Cupressoideæ, on the other hand, have indicated a considerable amount of group resistance to damping-off.

(6) The best control method appears to be the disinfectant treatment of the seed-bed soil before or immediately after seed is sown. Sulphuric acid has been found very useful for conifers, as they are apparently especially tolerant of acid treatment. No method has yet been worked out to a point at which all of its details are entirely

satisfactory, though the acid treatment has now been in successful use for several years at some nurseries. At most nurseries, if the minimum effective quantity of acid is used, there is no need of any special precautions to prevent injury to the seedlings. It is not expected that any single treatment can be found that can be universally applied without change in details irrespective of differences in soil characters and in fungous flora.

(7) *Corticium vagum* and *Fusarium* spp. have been previously shown to be parasitic on pine seedlings. Different strains of *C. vagum* are found to vary considerably in their ability to cause damping-off, certain strains being consistently destructive and others much less active in tests conducted on different species of pine and several years apart. The differences in activity between strains were greater, and apparently rather more constant from one experiment to the next, than with Peltier's strains in his carnation experiments. Comparison of the results on pine with those of Edson and Shapovalov on potato gives some indication that strains vigorously parasitic on one of these hosts are likely to be parasitic on the other also.

(8) *Pythium debaryanum*, reported on many hosts and proved to be parasitic on few, is shown by repeated inoculation, reisolation, and reinoculation to be capable of causing the damping-off of seedlings of pine species. The identity of the fungus causing the damping-off of conifers with that attacking dicotyledons has been established by cross-inoculations as well as by morphological comparison. Inoculations on unheated soil are much less destructive than on heated soil. *Pythium debaryanum* has been obtained in culture from *Picea engelmanni*, *P. sitchensis*, *Tsuga mertensiana*, *Pinus banksiana*, *P. nigra austriaca*, *P. ponderosa*, *P. resinosa*, and *Pseudotsuga taxifolia*. In addition, fenugreek (*Trigonella foenum-graecum*), cowpea (*Vigna* sp.), and rice (*Oryza sativa*) are reported as apparently new hosts among the dicotyledons. In inoculations the fungus has been successfully used on *Pinus banksiana*, *P. ponderosa*, *P. resinosa*, and in a preliminary experiment on *Pseudotsuga taxifolia*. It had already been successfully used in preliminary inoculations on *Picea canadensis* by Hofmann (77).

Differences in parasitic activity on pine are found between different strains of *Pythium debaryanum*. These differences are not as large and partly for this reason their constancy is not quite as conclusively demonstrated as in the case of the strains of *Corticium vagum*.

(9) *Rhizosporangium aphanidermatus* Edson, a parasite of radish and sugar beet, in many ways closely resembling *Pythium debaryanum*, has killed seedlings of *Pinus banksiana* and *P. resinosa* in certain experiments, and reisolations and reinoculations have been

made. The strain available is much less destructive to the pines than most of the *P. debaryanum* strains used, and as the fungus has never been isolated from coniferous seed beds it is not believed to be of any great importance in forest nurseries.

(10) *Phytophthora* sp. from *Pinus resinosa* seedlings has been successfully used in inoculation on *Pinus resinosa* and in a preliminary test on *P. ponderosa*. The strains available have been less destructive to the pines than *Pythium debaryanum* and the stronger strains of *Corticium vagum*. It is not common. Its relation to *Phytophthora fagi*, the European damping-off parasite of both conifers and dicotyledons, which has not been reported in this country, is being investigated.

(11) A fungus referred to *Pythium artotrogus*, also obtained from damped-off *Pinus resinosa*, has indicated a very low degree of parasitism on this host, even less than that shown by the Rheosporangium and Phytophthora strains. An addition is made to the statements in a previous paper concerning the ability of *Botrytis cinerea* to cause damping-off in conifers.

(12) The results of the cultural or direct examination of 742 disease foci in seed beds of various conifers are reported. *Pythium debaryanum* in the plate-culture examination method, considered more reliable than direct examination, appeared in 51 per cent of the foci from untreated beds, while *Corticium vagum* was found in 19 per cent. In foci in beds treated with various disinfectants, *P. debaryanum* was identified in 38 per cent of the foci and *C. vagum* in only 4 per cent. When direct microscopic examination was substituted for isolation, *C. vagum* was found on a larger proportion of the seedlings. It was not found at all in soil which had been heated. The relative ease with which it appears to be controlled by soil disinfection is in agreement with its poor adaptation for aerial distribution. It was found more commonly in cases in which the seedlings were directly examined than when cultures were made. In view of the fact that at least some of the Corticium foci extend rapidly and include very large numbers of seedlings, it seems that the Corticium may be as important as *P. debaryanum* in causing the damping-off of pines.

(13) *Fusarium* spp. have occurred more commonly in plate cultures than either of the above-mentioned fungi. Because little is known as to the parasitism of different species of this group on conifers, it is not possible to make any statement regarding the importance of the individual species. The evidence as a whole indicates so much importance for *Pythium debaryanum*, *Corticium vagum*, and for the *Fusarium* spp. considered as a group that no one of the three can be safely said to be more important than the others. None of the other fungi considered appear to be of real economic rank in the United States.

(14) In an inoculation experiment on the roots of pines 1½ months old, *Corticium vagum* and *Pythium debaryanum* were found able to cause the death of seedlings which had already developed rigid stems and to destroy the younger parts of the roots of seedlings which they were unable to kill. Indications were also obtained of similar but less vigorous action by *Fusarium moniliforme* and *F. ventricosum*.

(15) Data are given confirming the general belief that thick sowing favors the disease and indicating that soil acidity is, in general, unfavorable. Preliminary data on the relation of temperature and moisture to the disease are also presented. The parasitic activity of *Pythium debaryanum* in steamed soil was in one extensive test considerably decreased, following the inoculation of the soil with various saprophytes; this indicates both that competition of different fungi is a factor to be considered and that the inoculation of treated soil with saprophytes may sometimes prove of value in increasing the efficiency of heat disinfection. It is pointed out that with such a complex of parasites capable of producing identical symptoms on a number of different hosts, no relationship between environmental factors and the disease can be expected to hold in all cases.

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